assessed EGFR and erbB2 expression in rhabdomyosarcoma tumors and correlated our findings with histologic subtype.
Design: Sections from two tissue microarray blocks containing 66 rhabdomyosarcoma tumors (34 embryonal rhabdomyosarcoma (ERMS), 32 alveolar rhabdomyosarcoma (ARMS)) were surveyed by immunohistochemistry using antibodies specific for EGFR (Zymed; 1:10), and erbB2 (Dako, 1:50). EGFR and erbB2 immunostains were assessed for intensity (I) (0: no immunostaining; 1: weak; 2: moderate; 3: strong) and extent (E) (percent of 1000 neoplastic cells exhibiting membranous or membranous and cytoplasmic immunostaining). Expression of EGFR or erbB2 was considered positive if I x E >20. Correlations were assessed using the Chi-square test.
Results: Patients were 38 males and 28 females with a median age of 5.6 years (range 8 months - 19 years). Expression of EGFR was identified in 31/66 (47%) cases and correlated with ERMS subtype (26/34, 76%, vs. 5/32, 16%, $p<0.0001$). Expression of erbB2 was identified in 22/66 (33%) cases and tended to be more common in the ARMS subtype (13/32, 41%, vs. 9/34, 26%, $p=0.22$). Coexpression of EGFR and erbB2 was identified in 7 tumors, most of which (6/7) were ERMS. ARMS tumors were significantly more likely to lack expression of both EGFR and erbB2, compared to ERMS (16/32, 50%, vs. 5/34, 15%, $p<0.002$).

	ERMS	ARMS
EGFR-positive	26	5
EGFR-negative	8	27

	ERMS	ARMS
erbB2-positive	9	13
erbB2-negative	25	19

Conclusions: Expression of EGFR and/or erbB2 can be detected in a sizeable subset of rhabdomyosarcoma tumors. Notably, expression of EGFR appears to correlate strongly with ERMS, which also seems more likely to coexpress EGFR and erbB2.

Pulmonary

1400 Detection of the JC Virus Genome in Lung Cancers; Possible Role of the T-Antigen in Lung Oncogenesis

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Background: JCV virus is human polyomavirus. Subclinical infection with JCV occurs in 85-90% of the population worldwide. Primary infection in early childhood. Latency in the kidney and CNS. Reactivation when the immune system is impaired. JCV genome has early sequence that contains large T – and small t-antigen, viral late genes that contain viral capsid proteins (VP1, VP2 and VP3) and the regulatory region.
Design: We investigated the presence of its genome in 62 tumors, along with 23 samples of normal lung tissue, targeting the *T-antigen*, *VP* and *Agnoprotein* by nested PCR and Southern blotting followed by direct DNA sequencing. Copy number for the *T-antigen* was calculated by real-time PCR. Immunohistochemistry was performed to assess links between p53 and β-catenin in lung cancers and the presence of viral sequences.
Results: The *T-antigen* was detected in 25 of 62 lung cancers (40.3%) but only 4 of 23 normal lung samples (17.4%) (p=0.048), *VP* in 8 (12.9%) and 4 (17.4%) and *Agnoprotein* in 16 (25.8%) and 5 (21.7%). In total, the JCV genome was present in 33 of the lung cancers (53.2%) and 10 (43.5%) of the normal samples. Furthermore, the *T-antigen* was detected in 3 of the 4 cases with lymph node metastasis (75.0%) (p=0.042) and was more frequent in adenocarcinomas than in squamous cell carcinomas (p= 0.038). Viral DNA load was 37,743.1± 99,348.0 (mean ± SD) copies/μg DNA in cancers and 89.9 ± 68.6 copies/μg DNA in normal lung tissues. Immunohistochemistry showed significant correlations between *T-antigen* and p53 (p=0.022) and also nuclear detection of β-catenin (p=0.021).
Conclusions: It is concluded that the JCV genome might be integrated in cancer cells in approximately half of all Japanese lung cancer cases, and that the *T-antigen* may play a role in oncogenesis of lung cancers through inactivation of p53 and dysregulation of the Wnt signaling pathway.

1401 Microsatellite Instability Status in Giant Cell Lung Carcinoma

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Background: Giant cell carcinoma of the lung (GCC), an unusual morphologic variant of non-small cell carcinoma (NSCLC), is an aggressive epithelial neoplasm associated with poor survival. Recent published reports suggest an association between poor outcome in NSCLC and specific genetic alterations, such as (1) high levels of microsatellite instability reflected by loss of mismatch repair genes hMLH1 and hMSH2, or (2) estrogen receptor (ER) expression. However, in GCC their significance remains unclear. Our objective was to evaluate the clinicopathologic characteristics of GCC, to investigate the inactivation of the mismatch repair genes, and determine the expression of ER, PR and βHCG in GCC.
Design: We evaluated 14 patients with GCC treated with surgery at the Brigham and Women's Hospital between 1991 and 2005. We excluded one patient with preoperative neoadjuvant chemoradiation therapy. Clinical and pathologic features were evaluated in all cases. Pathologic stage was determined according to American Joint Committee on Cancer criteria (TNM stage). Immunohistochemical studies for expression of CK7, CK20, TTF-1 protein, βHCG, ER, PR, were performed in all cases. Expression of the mismatch repair (MMR) proteins hMLH1 and hMSH2 was evaluated by immunohistochemistry.
Results: The patients were 10 men and 4 women with a median age at diagnosis of 59 years (range 39 to 87). The mean tumor size was 4.35 cm (95 percent confidence interval, 2.7-6.0 cm). The TNM stage was I for 3 patients (21.4 %), II for 5 patients (35.7%), III for 3 (21.4%) and IV for 3 patients (21.4 %). The tumor cells were diffusely positive for CK7 in all cases and for TTF-1 in 50% of cases. βHCG was positive in one case (7%) and ER in one case (7%). The tumor cells did not express PR. CK20 was focally positive in 3 cases (21%). The expression of both hMLH1 and hMSH2 was present in all cases evaluated, indicating microsatellite-stable tumors.
Conclusions: Giant cell carcinomas of the lung are unusual, large, and aggressive tumors commonly associated with lymph node and distant metastases. The present study shows that MMR deficiency and thus high levels of microsatellite instability are not involved in the pathogenesis of giant cell lung cancer. Furthermore, tumor cells do not express ER, a reported marker of poor survival present in other lung cancers. Continued studies are needed to further define factors associated with poor survival in patients with giant cell lung carcinoma.

1402 E26 Transformation Specific (ETS-1) Does Not Correlate with Prognosis in Non-Small Cell Lung Cancers (NSCLC)

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Background: ETS-1 is expressed strongly in vascular endothelial cells and adjacent interstitial cells during angiogenesis, after which it is down-regulated. ETS-1 inhibition suppresses angiogenesis. ETS-1 has been identified in NSCLC and has been associated with tumor spread and prognosis in gastric and ovarian cancers. We evaluate tumor cell and blood vessel expression of ETS-1 in NSCLC.
Design: Tissue microarrays of 340 NSCLC with 5 year or greater follow-up were immunostained with ETS-1 (1:50, Novus Biologicals, Littleton, CO) using standard immunostaining techniques. Percentage of NSCLC cell nuclei and blood vessels expressing ETS-1 were scored on a scale of 0-3 (0=no staining; 1=<33% positive; 2=33-66% positive; 3=>66% positive). %). Intensity of nuclear staining was graded as negative, weak, moderate, or strong. Results were correlated with patient survival using Kaplan-Meier analysis.
Results: Endothelial cell nuclear positivity was observed within tumor blood vessels in 23/147 (16%); 9/147 (6%) showed tumor cell nuclear positivity; and 7/147 (5%) showed both. Staining intensity was uniformly weak, and present in fewer than 33% of the cell population. No association between endothelial cell or tumor cell staining and age, sex, smoking history, tumor stage, or survival was seen. Vessel staining tended to occur less often in predominantly bronchioloalveolar pattern adenocarcinomas (BA) compared to other NSCLC (p=0.2).
Conclusions: The majority of NSCLC in our series showed no tumor or blood vessel endothelial cell staining with ETS-1, and in the 16% of cases showing staining, ETS-1 positivity did not correlate with prognosis. The presence of ETS-1 expression suggests the potential for antibody-based treatment for ETS-1 positive NSCLC.

1403 Protein Gene Product (PGP) 9.5 Immunostaining Is Inversely Proportional to Tumor Grade in Squamous Cell Lung Carcinomas

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Background: PGP 9.5 is a ubiquitin hydrolase that is typically found in neuronal tissue, and the increased deubiquitination of cyclins by PGP 9.5 may allow for uncontrolled tumor cell growth. PGP 9.5 has been identified in non-small cell lung carcinomas (NSCLC), and one study has shown PGP 9.5 expressed more in high stage (stages 3 & 4) NSCLC than low stage (stages 1 & 2) NSCLC. We evaluated the expression of PGP 9.5 in NSCLC.
Design: Tissue microarrays of 340 NSCLC with 5 year or greater follow-up were immunostained with PGP 9.5 (1:40, Research Diagnostics, Concord, MA) using standard immunostaining techniques. Percentage of NSCLC cells expressing PGP 9.5 were scored on a scale of 0-3 (0=no nuclear staining; 1=<33% positive; 2=33-66% positive; 3=>66% positive). Intensity of nuclear staining was graded as negative, weak, moderate, or strong. Results were correlated with patient survival using Kaplan-Meier analysis.
Results: >10 % PGP 9.5 expression was noted in 5/88 (6%) squamous cell carcinomas and 26/183 (17%) adenocarcinomas. PGP 9.5 staining intensity (p=0.025) and percent staining (p=0.026) were found to be inversely related to tumor grade in squamous cell carcinomas. This relationship was not observed in other cell types; however, PGP 9.5 staining intensity was greater in squamous cell carcinomas than in adenocarcinomas (p=0.04). Neither staining intensity nor percentage of tumor cells staining with PGP 9.5 showed any significant relationship to survival.
Conclusions: PGP 9.5 is not specific for neuroendocrine neoplasms. The inverse relationship between PGP 9.5 staining and tumor grade in squamous cell carcinomas differs from the results of a prior study showing increased PGP 9.5 staining in higher-grade NSCLC. The staining pattern suggests the potential for antibody-based treatment focusing on early stage squamous cell NSCLC.

1404 Cox-2 and Angiogenic Factors in Neuroendocrine Lung Tumors

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Background: Cyclooxigenase-2 (Cox-2), a known mediator of inflammation, has been associated with angiogenesis and progression in several neoplasms. The relationship between Cox-2 and angiogenic factors, such as VEGF (vascular endothelial growth factor) and its receptor VEGFR2/Flk-1 in neuroendocrine lung tumors (NELT) is not well known.

Design: Tissue microarrays from 43 NELT (27 typical carcinoid –TC-, 7 atypical carcinoid –AC-, and 9 large cell neuroendocrine carcinomas -LCNEC) following surgery were stained with antibodies against Cox-2 (Cayman Chemical), VEGF (Neomarkers), and Flk-1 (Santa Cruz Biotech). Tumor cell positivity (percentage and intensity) was semi-quantitatively scored (range 0-300). Cases with score ≥30 were defined as positive. Immunohistochemical results were correlated, as well as with histologic type.
Results: Age of the patients ranged from 20 to 74 years (mean 51); 22 (51%) were men and 21 (49%) women. Cox-2 expression was seen in 42%, VEGF in 16%, and Flk-1 in 33% cases. A positive significant correlation was found between Cox-2 and VEGF (p=0.001), Flk-1 (p<0.000), and histology (30% TC, 43% AC and 79% LCNEC; p=0.04). Tumors showing VEGF also expressed Flk-1 (p=0.003), but the level of the staining among histological types differed significantly only for Flk-1 (22% TC, 29% AC and 67% LCNEC; p=0.047). Among the 34 carcinoid tumors, 7 had metastasis: 11% (3/27) were TC and 57% (4/7) were AC (p=0.02). However, no correlation between VEGF, Flk-1 or Cox-2 expression and the presence of metastases was found.
Conclusions: In the present series of NELT, our findings support the relationship between Cox-2 and angiogenesis in NELT. Moreover, the association of Cox-2 and Flk-1 with a more aggressive histologic subtype might be relevant for the selection of patients that may benefit of targeted therapeutic modalities.

1405 The Dilemma of "Asbestosis" or Lung Fibrosis – A Case-Referent Necropsy Study of 188 Asbestos Exposed Naval Dockyard Workers and 175 UK Non-Exposed "Control" Subjects

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Background: Accurate diagnosis of asbestosis is important for clinical and medicolegal reasons and is of use in lung cancer attribution. Controversy exists as to whether a lung cancer should be attributed to asbestos exposure in the absence of radiologically or histologically detectable fibrosis.Mild degrees of interstitial fibrosis (grades 1 to 2) are not usually detectable radiologically.At a simplistic level diagnosis of asbestosis rests on the presence of diffuse interstitial fibrosis and asbestos bodies but interpretive problems exist for both parameters.The aim of this study was to compare rates of the various grades of fibrosis in asbestos exposed and non-exposed subjects to assess the validity of the histological diagnosis of "early asbestosis".
Design: Fibrosis was graded by three observers according to the Roggli-Pratt method in at least three lung tissue sections prepared from post mortem samples obtained from 188 naval dockyard (Devonport) workers exposed to asbestos and 175 non-exposed UK subjects. The latter comprised routine post mortem cases from urban and rural areas who had died from diseases unrelated to asbestos.
Results:

	Grades of fibrosis (%) for dockyard workers and controls				
fibrosis grade	0	1	2	3	4
Dockyard (188)	56	12	8	9	16
Control(175)	59	23	12	4	2

The percentage rates for grade 0 were similar for both asbestos and non-asbestos exposed subjects. Grades 1 and 2 fibrosis were more frequent in the non-exposed group.Excess rates of fibrosis in the asbestos cohort were only apparent in grades 3 and 4. Fibroblastic buds were seen in less than 2% of the asbestos exposed cohort but were observed in all of the controls with grade 4 fibrosis.
Conclusions: Mild degrees of interstitial fibrosis (grades 1 and 2)are frequent in the non-asbestos exposed population. This should be kept in mind when assessing cases of potential asbestosis. Overinterpretation of mild degrees of interstitial fibrosis may lead to misdiagnosis of asbestosis and false attribution of lung cancer to asbestos exposure. The presence of fibroblastic "buds" suggest a diagnosis other than asbestosis.

1406 Array-Chromosomal Genomic Hybridization (CGH) Study Comparing Bronchioloalveolar Carcinoma and Invasive Lung Adenocarcinoma

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Background: Bronchioloalveolar carcinoma (BAC) is a subtype of lung adenocarcinoma (ADC) that has excellent prognosis. BAC lacks evidence of stromal, vascular or pleural invasion by histological definition. However, individual pathologist's interpretation for the presence of stromal invasion is subjective and could be variable. Furthermore, the invasive potential of BAC and the concept that BAC is a precursor of invasive adenocarcinoma remain to be proven. Based on metaphase-CGH studies showing association of genomic imbalances with tumor progression, we hypothesized that genomic profile changes may distinguish BAC from the invasive ADC, even those with prominent BAC-like growth pattern.
Design: We used high-density human bacterial artificial chromosome (hBAC) array-CGH to characterize the genomic changes of 14 BACs, 6 BACs with minimal invasion, and 9 invasive ADC with prominent BAC-like growth pattern (>90% BAC). 8 samples of normal lung tissue were studied as normal controls. DNA was extracted from archival formalin-fixed paraffin-embedded tissues and hybridized on the "27 K" SMRT (Sub Megabase Resolution Tiling set) array CGH. The results were analyzed using multiple computational software tools that have been developed for array CGH and microarrays.
Results: BACs showed a pattern of subtle but common genomic changes that distinguished them from BACs with minimal invasion and invasive ADC, by lower number of chromosomal changes and less variability. Gene copy gains were noted most commonly at 21q, 17q, 16q, 18q, 9q and 20q. Genomic changes recorded for either group were mostly in the sub-telomeric region of chromosomes. Several genes are novel candidate diagnostic markers for more precise classification of BAC associated tumors.
Conclusions: BACs can be distinguished from minimally invasive BACs and invasive ADC with prominent BAC-like growth pattern based on their genomic profile. The putative marker genes identified require further validated by other techniques.

1407 c-myc and pGSK-3beta Expression in Lung Carcinomas

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Background: Activation of the c-*myc* gene is a common feature of many neoplasms. Its upregulation has been implicated in tumor progression and metastasis in lung, breast and colon cancers. Glycogen synthase kinase-3 (GSK-3) is a proline-directed serine-threonine kinase whose overexpression enhances the phosphorylation and ubiquitination of c-myc protein. It has been shown that oncogenic K-ras stimulates Wnt signaling in colon cancer through inhibition of GSK-3beta. We evaluated the expression of c-myc and GSK-3beta in NSCLC and their relationships to patient survival.
Design: Tissue microarrays containing triplicate punch samples of 340 cases of NSCLCs were immunostained for c-myc (1:1500, Novus Biologicals, Littleton, CO) and phospho-GSK-3beta (1:25, Cell Signaling, Beverly, MA). Staining intensities were graded as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong) and the percentages of tumor cells staining were approximated to the nearest 10%. For each tumor, mean values for staining intensity and percentage were calculated, and used to derive an overall score of 0-3. Correlations with patient survival and histologic cell type were analyzed using Spearman rank correlation and Kaplan-Meier analysis.
Results: Overexpression (strong staining, 3+) and lack of expression (negative) of c-myc showed a trend for poor survival (p=0.9) however, the longest survival by Kaplan-Meier analysis was generally associated with intermediate staining intensity of c-*myc* . Expression or absence of p-GSK-3beta showed a negative correlation with survival.
Conclusions: C-myc and pGSK-3b are widely expressed in NSCLCs. PGSK-3b has not been studied in detail in lung cancer however, it is an important element in the WNT pathway. Variations in the level of expression patterns of these genes seems to show a relationship to prognosis. Pharmacologic modulation of their activities may represent a potentially beneficial approach to the treatment of NSCLC.

1408 Gene Expression Profiling of Microdissected Pulmonary Adenocarcinoma and Bronchioloalveolar Carcinoma

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Background: The main histologic feature differentiating bronchioloalveolar carcinoma (BAC) from other adenocarcinoma subtypes is the presence of tissue invasion. Acquisition of an invasive phenotype is critical in the progression of lung adenocarcinoma. We hypothesize that analysis of gene expression profiles of BAC and mixed subtype tumors (containing both invasive adenocarcinoma and BAC components) will reveal gene signatures associated with invasion.
Design: Frozen tissue was acquired from 12 BAC tumors and 14 mixed subtype tumors. Tumor cells were isolated from each sample with PALM Zeiss laser-capture microdissection. One sample was microdissected in triplicate to confirm the reproducibility of the protocol. Probe was produced by modified Eberwine protocol and was hybridized to Affymetrix U133 Plus 2.0 arrays. Data were analyzed using BRB array tools software and probe level normalization.
Results: Unsupervised clustering of the 26 samples resulted in the formation of three main clades. One of these clades contained the majority of the BAC samples (9 of 13 tumors in this clade were BAC). The other two clades contained predominantly mixed subtype tumors (only 3 BAC tumors clustered with the 13 tumors comprising the combined samples of the two predominantly mixed subtype clades). This separation was statistically significant (chi-square<0.05). The tumor that was dissected in triplicate showed a reproducible molecular signature that was reflected in the tight clustering of these samples in one part of the dendrogram.
Conclusions: Microdissection of BAC tumors and mixed subtype tumors reveals a molecular profile that allows for unsupervised clustering that correlated with the presence or absence of invasive phenotype. The method used to create the molecular profile is reproducible based on the performance of triplicate dissection.

1409 Inflammation of Airway Smooth Muscle in Allergic Asthma: An Ulstrastructural Approach

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Background: In asthma, the airway smooth muscle (ASM) is infiltrated by a variety of inflammatory cells that may directly interact with smooth muscle cells (SMC), influence their function and contribute to their remodelling but little is known about such interaction. We perform a morphometric analysis of ASM from asthmatic patients using electron transmission microscopy and seek direct interactions between SMC and inflammatory cells.
Design: 11 subjects with asthma and 7 normal controls were recruited for the study and underwent fiberoptic bronchoscopy. ASM was assessable in the biopsy specimens from 8 patients with asthma and 6 normal controls that were processed for electron microscopy. ASM bundles were orientated either in a longitudinal plane either in a transversal plane either tangentially. Computerized photographs and measures were performed by using the Analysis Soft Imaging System.
Results: Mean SMC size and mean SMC basal lamina thickness were larger in asthmatic patients than in controls (9.71 vs 6.93 µm, p=0.02 and 1.54 vs 0.69µm, p=0.02, respectively). In asthmatics, extra cellular matrix was frequently organized in large bland amounts of collagen deposits between SMC. ASM was infiltrated by mast cells, lymphocytes, monocytes/macrophages and rare neutrophils. Close contacts were observed between activated mast cells and SMC, and to a lesser extent between lymphocytes and SCM. Myofibroblast were observed in 6 out of 8 asthmatic patients between SMC bundles and some of them displayed close contact with mast cells and SMC.

Conclusions: Electron microscopy analysis demonstrates that, in asthma, there is a marked remodelling of ASM with an important deposition of collagen. SMC are in close contact with activated mast cells, lymphocytes and myofibroblasts that may play a direct role in such a phenomenon.

1410 Diagnostic Value of Routine Histochemical Organism Stains in the Work-Up of Granulomatous Inflammation Detected in Transbronchial Biopsy Specimens

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Background: Granulomatous inflammation is a common finding in bronchoscopic biopsy specimens, and the typical work-up includes histochemical organism stains in order to exclude an infectious process. These stains require additional laboratory resources, may delay case sign out, and the diagnostic yield of these stains is usually low. We sought to determine the diagnostic value of these stains in our practice.

Design: Retrospective review of 90 consecutive transbronchial biopsy specimens that demonstrated granulomatous inflammation as the principal histologic finding. The results of histochemical organism stains (acid-fast and fungal) were compared to the corresponding clinical microbiology results, clinical histories, and radiographic findings.

Results: 81 cases (81/90) were organism-negative by histochemical stains. Of these 81 cases, 6 cases of Mycobacterium avium-intracellulare and one case each of Pneumocystis, Blastomyces, and non-tuberculous mycobacterium were discovered by microbiologic techniques performed on paired biopsy and/or lavage samples. Of the 9 histochemical organism-positive cases, 8 demonstrated fungal organisms and one showed acid-fast bacilli. The histochemical stain was the only diagnostic evidence of infection in three of these cases (one case each of Histoplasma, PCP, and Blastomyces), for an overall diagnostic yield of 3/90 (3.3%). The single case of histochemical AFB-positivity was also detected and further characterized by microbiologic techniques and thus was not the sole diagnostic evidence of infection.

Conclusions: Although of relatively low yield, routine staining for fungal organisms should be done on every case of granulomatous inflammation, as direct smear and culture may occasionally give false-negative results. Routine AFB staining did not increase diagnostic yield in this series, and in fact was rather insensitive compared to microbiologic examination, especially in the case of MAI infection. Therefore, histochemical staining for AFB may be more appropriate in selected high risk cases rather than as a routine stain.

1411 Thymoma: An Immunohistochemical Study with Emphasis on CD5, Calretinin, and Lymphoid Markers

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Background: The immunohistochemical profile of thymomas is complex due to the variety of growth patterns and background lymphoid infiltrate. We studied a series of thymomas to further delineate type specific immunophenotypes with a focus on epithelial and lymphoid characteristics.

Design: A single institutional series of 15 sequential thymomas in the past 5 years were retrospectively identified, reviewed, and classified using a modified WHO system. Immunohistochemical profiles with emphasis on epithelial and lymphoid markers were performed and semiquantitated using commercially available antisera.

Results: The patients were 10 men and 5 women, with a mean age of 63.4 years. Two patients had myasthenia gravis. There were 2 type A, 5 type AB, and 8 type B tumors resulting in 7 "A" areas and 14 "B" areas. Type B thymomas were further subclassified as: B1 - epithelioid cells scattered in a dense lymphoid stroma or B3 - epithelioid sheets of cohesive cells. Epithelial markers CK7 and EMA were present in the majority of tumors. CK 7 was positive in 2 type A, 2 type AB and 3 type B tumors (B1 = 1 and B3 = 2). At the same time EMA was positive in 1 type A, 2 type AB and 4 type B tumors (B1 = 1 and B3 =3). Hence these markers did not determinately distinguish between type A and B. CK7 was however expressed in a greater proportion of "A" tumor cells (p<.01). Tumor staining for calretinin was positive, occurring more frequently in type A than type B thymomas (p=.01). Regarding lymphoid markers CD5 was absent in A tumor cells and more frequent in B3 (67%) than B1 (14%, P=.01) tumor cells. Bcl-2 was present in 100% of B3 tumor cells vs. 13% of B1 and 14% of A (p=.02). CD57 was expressed in 71% of type A and 45% of type B thymomas. Background lymphoid infiltrate in B thymomas was stronger with CD99 than CD1a. B-cell follicles were present in the stroma of 3 B type thymomas; AB, B1 and B3 tumors respectively. Tumor staining for vimentin was mostly negative resulting in noncontributory results.

Conclusions: We conclude that B3 thymomas are characterized by CD5 and Bcl-2 positivity indicating that CD5 is not a reliable marker of thymic carcinoma as previously reported. Calretinin characterizes type A thymomas and is therefore not a reliable marker to distinguish them from mesothelial proliferations. CD99 is a better marker than CD1a for immature thymoma lymphocytes. B cell follicles are not uncommon in type B thymomas and do not indicate a separate tumor subtype.

1412 Pathologic Evaluation of Invasion in Pulmonary Adenocarcinoma

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Background: Current staging of lung adenocarcinomas is based on gross measurements of tumor size and pleural invasion. With the recognition that the non-invasive growth pattern of bronchioloalveolar carcinoma is clinically significant, we hypothesized that quantitation of invasion within mixed subtype adenocarcinoma would provide prognostic information.

Design: All slides from resected primary lung adenocarcinomas (180 patients) at Columbia-Presbyterian during 1997-2000 were reviewed and classified, focusing on histologic subtypes, gross size, node status, invasive growth patterns, vascular invasion and extent of bronchioloalveolar growth. Tumors were classified as bronchioloalveolar carcinoma (BAC), mixed subtype adenocarcinoma (AC) with BAC component, or invasive AC without BAC component. In all cases, the largest linear extent of invasive growth was measured histologically. In cases under 2.5 cm this measurement was used to determine invasive size; for predominantly invasive tumors (<50% BAC growth pattern) larger than 2.5 cm, the gross size was considered equivalent to invasive size for the analysis.

Results: Of the 180 patients, 70 were men and 110 were women, with a mean age of 66.5. The histologic groups included pure BAC (n=9), predominant BAC with < 6.0 mm of invasion (n=22), mixed subtype AC with BAC component (n=88), and pure invasive AC (n=61). Bivariate correlation analysis of all cases identified a relationship of invasive size and of vascular invasion with nodal metastasis (Spearman correlation r=.277, p<.0001, and r=.505, p< .0001, respectively). Within BAC and mixed AC subgroups (BAC pattern containing subgroups), invasive size and vascular invasion were also correlated with nodal metastasis (Spearman correlation r= .315, p< .0001 and r= .46, p< .0001, respectively). Gross size measurement did not correlate with nodal metastasis in either analysis

Conclusions: In primary resected adenocarcinomas, measured extent of invasive growth and vascular invasion are correlated with nodal metastasis, while measurement of gross size alone is not. This suggests that in tumors with a significant proportion of bronchioloalveolar growth, a histopathologic quantitation of invasive growth may more accurately reflect metastatic potential.

1413 Diffuse Pulmonary Neuroendocrine (NE) Cell Hyperplasia, Tumorlets and Carcinoid Tumors: A Clinicopathologic Study of 22 Cases

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Background: Only recently has diffuse idiopathic pulmonary NE hyperplasia been recognized as a preinvasive lesion for a rare subset of pulmonary carcinoid tumors. Due to the rarity of this condition, little is known about its clinical features and the spectrum of pathologic features. 22 cases were reviewed to better define this problem.

Design: Cases were retrieved from the files of the authors. The slides were reviewed and in selected cases immunohistochemistry was performed for chromogranin, synaptophysin, CD56 and TTF1. Airways were assessed for the presence of fibrosis. Wedge biopsies and lobectomies from 14 and 8 patients respectively were available for review including specimens from multiple lobes in 13 patients.

Results: All 22 patients were women with a mean age of 65 years (41-78 years). Of those with smoking histories, 7 were nonsmokers, 5 were former smokers and 3 were active smokers. Most cases were discovered as incidental radiologic findings during evaluation for other reasons, particularly followup of other malignaicies. Nine patients had a previous history of other types of cancer breast (n=4), melanoma (n =2), endometrium (n=2), lung (n=1), bladder (n=1). Respiratory symptoms included cough (n=4), chest pain (n=2) and dyspnea (n=1). Radiologic findings consisted most often of multiple pulmonary nodules (50%) that represented a spectrum of tumorlets and carcinoid tumors or a solitary nodule (41%) that represented a carcinoid tumor. No nodules corresponding to tumorlets or carcinoid tumors were seen radiographically in the remaining cases (9%). Multiple carcinoid tumors (up to 6) were found in 9 cases; a single carcinoid tumor was present in 9 cases and 4 patients had only tumorlets and NE hyperplasia. NE markers highlighted more NE hyperplasia than appreciated on H and E. TTF1 was positive in tumorlets and carcinoid tumors. All carcinoid tumors were typical except for one case of atypical carcinoid that proved fatal. The only other fatality was a patient who died of lung cancer. All other patients remain alive as of last followup (mean 4 years, range 0.25-12 years).

Conclusions: Diffuse pulmonary neuroendocrine cell hyperplasia with tumorlets and carcinoid tumors characteristically occurs in middle aged to elderly women. These patients frequently have one or more carcinoid tumors and most have a favorable outcome.

1414 High-Density Tissue Microarray-Based Appraisal of the Prognostic Value of p38 and phosphor-p38 in Non-Small Cell Lung Cancers

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Background: The p38 mitogen-activated protein kinase (MAPK) signal transduction pathway plays an essential role in regulating many cell processes and is selectively activated in non-small cell lung cancer (NSCLC). This high-throughput microarray study was performed to assess the relationship between expression of p38 and its phosphorylated form, phosphor-p38, to prognosis in NSCLC.

Design: Immunohistochemistry for p38 (1:10, DakoCytomation, Carpenteria, CA) and phosphor-p38 (1:25, Cell Signaling, Beverly, MA) was performed using standard avidin-biotin techniques on high-density tissue microarray slides containing samples from 346 NSCLCs with more than five years of clinical follow-up. Staining was scored semi-quantitatively as follows: 0=absent staining, 1 + staining = 1-32%, 2 + staining = 33-66%, 3 + staining >66% of tumor cells staining. Information about patient survival and tumor stage was collected. Statistical analysis was performed using Kaplan-Meier analysis and Spearman Rank correlation.

Results: Staining of NSCLC for p38 was as follows: 125 (36%) negative, 86 (25%) 1+, 64 (18%) 2+, 73 (21%) 3+. For phosphor-p38, results were: 162 (47%) negative, 118 (34%) 1+, 58 (17%) 2+, 8 (2%) 3+. p38 expression correlated with phosphor-p38 expression, but no significant correlation was observed between expression of either of these markers and histologic subtype, grade, stage, or gender.

Conclusions: The wide expression of p38 and phosphro-p38 in NSCLCs may indicate a role for these proteins in the pathogenesis of these neoplasms. However, there does not appear to be a correlation between histologic subtype, grade, stage, or gender in NSCLC and p38 and phosphor-p38 expression.

1415 Performance Characteristics for a Diagnostic Molecular Test for EGFR Gene Mutations, KRAS Mutations, and EGFR Amplification in Lung Adenocarcinomas

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Background: EGFR mutations are thought to be present in approximately 10% of lung cancers, with some studies suggesting up to 40% prevalence. Sequence mutations do not occur in other types of tumors. KRAS mutations are also found in approximately 30% lung carcinomas, and are mutually exclusive with EGFR mutations. The presence of these mutations has been suggested to have prognostic and therapeutic implications.

Design: All cases of adenocarcinoma that were processed at the University of Pittsburgh for a six month period (February through August 2005) were analyzed for mutations in EGFR exons 19 and 21, KRAS exon 1, and for EGFR gene amplification using FISH. For the PCR-based testing of EGFR and KRAS, tumor sections with adjacent normal tissue were microdissected and DNA was extracted. Cycle sequencing using an automated sequencer was performed (BigDye Terminator kit and ABI Prism 3100 instrument). EGFR FISH was performed using standard protocols and analyzed for amplification.

Results: A total of 59 patients, 37 female (63%) and 22 male (37%), with non-small cell lung carcinoma between ages 41 and 84 (Female/ Male Ratio: 1.68, Median Age; Total: 69, Female: 68, Male: 66.5) were studied. The type of tumors were; 56 adenocarcinoma, 3 adenosquamous carcinoma. The majority of the cases were stage I (64%) and stage II (25%), followed by stage IV (7%), stage II (2%). EGFR mutations were rare in our series, with only 6.7% of patients exhibiting an exon 19 deletion mutation and no exon 21 point mutations. KRAS mutations were more common, 27.1% with codon 12 mutations. Amplification of the EGFR gene was seen in 6 cases, none of which had the EGFR sequence mutation.

Conclusions: Our results show that in lung carcinomas EGFR E19 mutations are rare (4 cases; 6.77%), E21 were not seen, KRAS is common (16 cases; 27.11%), and EGFR amplification is rare (7 cases; 11.86%). In our clinical practice, although the finding of EGFR mutations has significance for treatment, a staged approach for molecular testing, with KRAS sequencing as the first step, would improve the cost-effectiveness and turnaround time for this assay.

1416 Over-Expression of Squamous Cell Carcinoma Antigen (SCCA) in Idiopathic Pulmonary Fibrosis

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Background: Idiopathic pulmonary fibrosis (IPF), characterized histopathologically by the usual interstitial pneumonia (UIP), is a progressive disease with 5-year survival less than 50%. A few molecular tissue markers have been studied in IPF in order to clarify the etiopathogenetic mechanisms leading to the disease. Squamous cell carcinoma antigen (SCCA) is a serin protease inhibitor (serpin) physiologically found in the spinous and granular layers of normal squamous epithelium and typically expressed by dysplastic and neoplastic cells of epithelial origin. No information is actually available on its expression in IPF.

Design: In the present study we analyzed the immunohistochemical expression of SCCA in lung samples from 37 IPF patients (17 open lung biopsy and 20 explanted lungs, mean age was 55±8.8 yrs) and 10 non-implanted donor lungs as control. A quantitative assessment of SCCA expression was undertaken in each IPF sample by counting at least 500 alveolar epithelial cells. The extent of fibroblastic foci was assessed in all open lung biopsies. Clinical data including lung function parameters (forced expiratory ventilation, FEV, forced vital capacity, FVC, carbon monoxide diffusing capacity, DL_{CO}) were available in all cases.

Results: SCCA was expressed in bronchial basal cells in both IPF and control cases. Alveolar epithelial cells in IPF cases showed a strong nuclear and/or cytoplasmic expression. A more marked expression was noted in cuboidal or dysplastic epithelial cells. Alveolar SCCA expression was not detected in any of control cases. A direct correlation was found between SCCA expression and the clinical duration of the disease ($p<0.001$). SCCA was significantly more expressed in patients with short clinical duration of the disease (< 12 months) than in patients with a longer or end-stage disease ($p=0.004$ and $p<0.001$ respectively. No correlations were observed between SCCA expression and the extent of fibroblastic foci and other clinical parameters.

Conclusions: The expression of SCCA in the alveolar epithelium corroborates the hypothesis that disturbed or abnormal epithelial alveolar regeneration is an important step in the pathogenesis of IPF. Alveolar epithelial instability is a pathological process involved progressively in the clinical history of the disease.

1417 CT Screening for Lung Cancer: Comparison of Pathologic Findings of Baseline to Annual Repeat Cancers

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Background: Screening for cancer is a periodic, repetitive process beginning with baseline screening followed by periodic screening. The cancers detected at baseline may differ from those found at subsequent screens in their biology as reflected in their histologic appearances and stage.

Design: Screenees included 9,727 with baseline CT and 10,921 annual repeat CT screenings among smokers who were considered fit to withstand thoracic surgery should any cancer be found. A total of 250 diagnoses of lung cancer were made, 199 baseline screen-diagnosed and 46 annual repeat screen-diagnosed cases; 198 were resected. There were 5 interim diagnoses in the baseline cycle. The pathologic and histologic features of the resected cases were ascertained and reviewed by the pathologists. At the time of resection, 86% of the baseline cancers and 81% of the repeat cancers had no lymph node or other metastases.

Results: Adenocarcinomas made up 75% of the baseline screen-diagnosed cases and Squamous cell carcinomas , and small cell carcinomas made up 9% and 5% respectively. Typical carcinoids were only found in the baseline cases and were 7% of them. Among the repeat screen-diagnosed cases, 50% were adenocarcinomas, 20% were Squamous cell carcinomas and 17% were small cell carcinomas. Multiple carcinomas were found in 19% of baseline and 14% of repeat -diagnosed cases; most were adenocarcinomas. Bronchioloalveolar carcinomas were found in the baseline cases, but not as index cancers among the repeat cancers; all were subsolid nodules on CT scan.

Conclusions: Differences were found between CT baseline and repeat CT screen-diagnosed carcinomas, but most cases in both groups were resectable and at low stage.

1418 NFkB Expression in Neuroendocrine Neoplasms of the Lung

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Background: Chemotherapeutic treatment of pulmonary small cell carcinoma (SCC) has a complete response rate of 50 to 60% when the tumor is of limited stage, but only 15-20% with advanced disease. One potential mechanism for chemoresistance involves the activation of NFkB by chemotherapeutic agents, which promotes cell survival through anti-apoptotic effects. We evaluated NFkB expression in high grade and low grade neuroendocrine neoplasms.

Design: Tissue microarrays were constructed from 45 SCCs, 11 large cell neuroendocrine carcinomas (LCNECs) and 54 typical carcinoid tumors (CARs), using three samples of formalin-fixed paraffin-embedded tumor tissue from each case. Immunohistochemistry for NFkB (1:100, South Signaling, Beverly MA) was performed on recut sections of the microarrays. The percentage of tumor cells with nuclear staining was scored as 1 = <33%, 2 = 33-66%, 3 = >66%, and nuclear staining intensity was graded as 0 = negative, 1 = weak, 2 = moderate and 3 = strong.

Results: Only one tumor in each category expressed NFkB (SCC= 1/45, LCNEC 1/11, CAR 1/54).

Conclusions: NFkB is not overexpressed in the majority of low grade or high grade neuroendocrine tumors of the lung, so its effects are unlikely to account for chemoresistance in this group of neoplasms.

1419 Expression of SHIP-1, PAX-5, p-AKT and FKHR in 344 Non-Small Cell Lung Carcinomas with Long-Term Follow-Up: Relationship to Survival

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Background: For non-small cell lung cancers (NSCLCs), accepted prognostic factors of tumor stage and pretreatment performance status do not completely account for variation in survival. SHIP-1, PAX-5, p-AKT and FKHR are regulators of cell growth and apoptosis that represent logical candidate molecules to evaluate for their relationships to prognosis. SHIP-1 negatively affects cell proliferation by increasing G1 transit time. The PI3K/p-AKT pathway promotes proliferation and increased cell survival. Downstream regulation of AKT results in reduction of pro-apoptotic factors and inhibitors of cell cycle proteins and genes such as FKHR, PTEN, p27, fas ligand, Bim, Bcl-6 and caspase 9. PAX-5 is involved in transcriptional control of tissue development and cell differentiation.

Design: Tissue microarrays (TMA) comprised of triplicate punch samples from 344 NSCLC were immunostained for SHIP-1 (1:100, Lab Vision), PAX-5 (1:200, Santa Cruz), p-AKT (1:50, Santa Cruz) and FKHR (1:300, BD Transduction). For each punch sample, the percentage of tumor cells staining was scored as follows: 0=no staining, 1 = <33%, 2 = 33-66%, 3 = >66%. Staining intensity was graded as 0 = negative, 1 = weak, 2 = moderate, 3 = strong. For each tumor, mean values for staining intensity and extensiveness were calculated and used for statistical analysis. Five-year survival data and tumor stage were collected for all subjects, and statistical analysis was performed using Kaplan-Meier analysis, the Kruskal-Wallis test, the Mann-Whitney U-test, and the Least Significant Difference test.

Results: Squamous cell carcinomas showed significantly higher p-AKT ($p=0.02$) and PAX-5 ($p<0.0001$) expression than the other histologic types. In early stage NSCLCs, tumor expression of FKHR was associated with a statistically significant 5-year survival advantage ($p=0.04$), and a trend towards improved survival was also noted for all FKHR-expressing NSCLCs as a group ($p=0.09$). Expression of SHIP-1, PAX-5 and p-AKT showed no statistically significant relationship to 5-year survival.

Conclusions: Expression of FKHR may predict improved survival in NSCLCs, particularly in early stage neoplasms.

1420 Pneumocytic Adenomyoepithelioma

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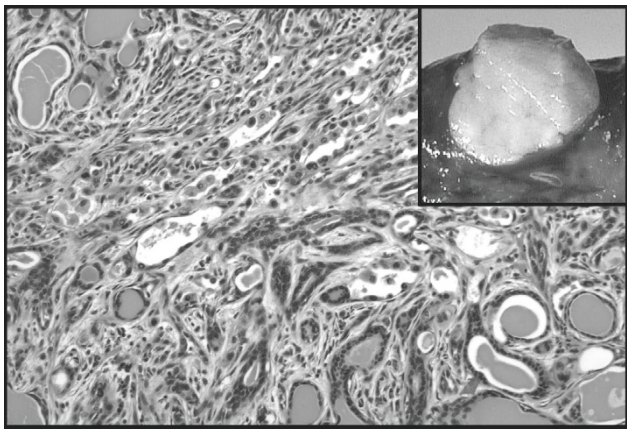
Background: Pulmonary tumors with dual epithelial and myoepithelial differentiation are rare, thought to be of bronchial gland origin and classified similar to salivary gland neoplasms. We report a series of a distinctive subtype of adenomyoepithelial tumors, which also show pneumocytic differentiation.

Design: The first case was identified and characterized ultrastructurally at University of Chicago in 2003, and since then 4 additional cases were collected from other institutions. Histochemical and immunohistochemical studies were performed in all tumors.

Results: All 5 patients were females, 52-63 years old. In contrast to most salivary gland type pulmonary neoplasms, no tumor arose in large airways; rather all were located peripherally. All tumors were grossly circumscribed, measuring 0.8-2.6 cm, and histologically showed biphasic, glandular and spindle cell differentiation. The glands were filled with colloid-like secretion and had an inner epithelial cell layer with pneumocytic characteristics (pankeratin, EMA, TTF-1 and surfactant positive), surrounded by an outer layer of myoepithelial cells merging with foci of spindled myoepithelial cells (HMWK, S100, SMA, calponin, caldesmon and p63 positive). Alveolar entrapment was excluded histologically and by absence of elastic fibers in the tumor. Ultrastructurally, many of the epithelial cells exhibited pneumocytic characteristics including cytoplasmic lamellar bodies. The outer layer cells and the spindle cells contained thin filaments with focal densities, confirming myoepithelial differentiation.



Conclusions: This is the first reported series of a distinctive lung tumor with epithelial, myoepithelial and pneumocytic differentiation, proven immunohistochemically and ultrastructurally. It differs histologically from all previously recognized pulmonary salivary gland-type and pneumocytic tumors. It is a unique benign appearing neoplasm for which the designation pneumocytic adenomyoepithelioma is appropriate.

1421 Clinicopathologic Features of Long-Term Survivors after Extrapleural Pneumonectomy in Patients with Diffuse Malignant Pleural Mesothelioma

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Background: Malignant pleural mesothelioma (MPM) is an uncommon malignancy with a poor median survival ranging from 4 to 18 months in various series. Although there is no uniformly accepted standard therapy, the best-documented potentially curative approach has been extrapleural pneumonectomy, followed by chemotherapy and radiotherapy (trimodality approach) in selected patients. However, clinicopathologic characteristics of patients with malignant pleural mesothelioma with long-term survival remain poorly defined, unclear, and often attributed to patients with well-differentiated and/or localized subtypes.

Design: We studied 18 patients with malignant pleural mesothelioma treated with extrapleural pneumonectomy followed by adjuvant chemotherapy and radiation between 1984 and 1993 who survived longer than the 75th percentile (range 39 to 100 months) on the basis of an earlier investigation. The mean follow-up period was 61.1 months. In the surgical specimens, tumor status was classified with the Tumor-Node - Metastasis staging system of the American Joint Committee on Cancer. We evaluated various clinicopathologic parameters that might aid in patient selection for this type of therapy and predict outcome in this patient population. Quantitative digestive studies for asbestos bodies were evaluated in 94% of cases.

Results: The patients were 12 men and 6 women with a median age at diagnosis of 47 years (range 31 to 64). Mesothelioma status was determined in each extrapleural pneumonectomy specimen and tumor was sampled for histological evaluation in an average of 46 slides (95 percent confidence interval, 34.1-58.7). The TNM stage was I for 4 patients (22.2 %), II for 4 patients (22.2%) and III for 10 (55.5%). Sixteen patients had epithelial mesothelioma and two had mixed, epithelial and sarcomatoid mesothelioma. Among the epithelioid tumors, two (11.1%) were well-differentiated mesotheliomas. The average asbestos count was 1332 asbestos bodies/gram of wet lung.

Conclusions: Our results suggest that younger patients treated with trimodality approach have better overall survival. Stage and differentiation does not appear to be a factor, as the majority of cases were stage III. Continued studies are needed to further define factors associated with long-term survival in patients with malignant pleural mesothelioma treated with extrapleural pneumonectomy followed by chemotherapy and radiotherapy.

1422 Sarcomatoid Malignant Mesothelioma: Immunohistochemical Characteristics of 24 Cases

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Background: Numerous published analyses have described the immunohistochemical characteristics of epithelioid malignant mesothelioma. However, immunohistochemical analyses of the less common type, sarcomatoid mesothelioma are limited and its distinction from sarcomatoid carcinoma, various sarcomas and other tumors of the chest wall, lung and pleura is often problematic. We report immunohistochemical characteristics in a series of 24 cases of sarcomatoid mesothelioma, using 9 commercial antibodies with potential for distinguishing sarcomatoid mesothelioma from other spindle cell tumors.

Design: We evaluated 24 patients with pleural sarcomatoid mesothelioma who had surgery (12 extrapleural pneumonectomies, 9 pleurectomies and 3 large biopsies) performed between 1989 and 2005 at the Brigham and Women's Hospital. Pathologic diagnoses of sarcomatoid mesothelioma were confirmed and clinicopathologic features and demographic data recorded. Immunohistochemical studies for AE1/AE3, CAM5.2 and MNF-116 keratins, calretinin, WT-1 protein, bcl-2, CD-34, D2-40 and podoplanin were performed on sections cut from formalin-fixed, paraffin-embedded blocks of tumor. Cases with at least 1% immunoreactive tumor cells by light microscopy were reported as positive and those with < 1%, negative.

Results: The patients were 23 men and 1 woman with a median age at diagnosis of 64.7 years (range 47 to 76). Tumor was sampled for histological evaluation in an average of 22 slides (95 percent confidence interval, 17.2-27.3). Tumor cells were positive for the keratin proteins AE1/AE3 in 24/24 cases, CAM 5.2 in 24/24 cases and MNF-116 in 21/21 cases. Calretinin was positive in 6/24 cases, WT-1 (nuclear) in 8/24 cases, bcl-2 in 0/24 cases, CD-34 in 0/24 cases, D2-40 in 24/24 cases and podoplanin in 24/24 cases.

Conclusions: This study shows that sarcomatoid mesothelioma has a characteristic immunohistochemical profile. Tumor cells were positive for keratin proteins, D2-40 and podoplanin and were negative for bcl-2 and CD-34. This panel of antibodies may be helpful in distinguishing sarcomatoid mesothelioma from other tumors. In our study, D2-40 and podoplanin are highly sensitive immunohistochemical markers for sarcomatoid mesothelioma. Additional studies are required to define their role in the differential diagnosis of other spindle cell tumors.

1423 Characterization of Epidermal Growth Factor Receptor (EGFR) Gene Copy Number by Chromogenic In Situ Hybridization (CISH) in Atypical Adenomatous Hyperplasia, Bronchioloalveolar Carcinoma and Adenocarcinoma of the Lung

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Background: Atypical adenomatous hyperplasia (AAH) is postulated to be the earliest precursor lesion in lung carcinogenesis. The epidermal growth factor receptor (EGFR), one of the members of the Erb-2 family of receptors is commonly expressed in non-small cell carcinoma of the lung (NSCLC). A subset of the patients with NSCLC has molecular abnormalities in the EGFR gene, including missense mutations and deletions and/or abnormal gene copy numbers, and the relative importance of each of these to patient outcome is an area of great interest. Recent reports show that EGFR mutations are rare or absent in AAH and are rare in bronchioloalveolar carcinoma (BAC). However, the EGFR gene copy number status in AAH is unknown.

Design: We examined the EGFR gene copy number status in lung adenocarcinomas (10 cases) and synchronous AAH (9 cases) in the surgical pathology specimens resected from ten patients; and BAC from twelve patients. EGFR gene copy number was analyzed by CISH using paraffin embedded tissue sections and EGFR probes as recommended by the manufacturer (Zymed Laboratories Inc.). Sections of a known positive case of high-grade glioma were used as positive control. In addition, we correlated the gene copy number with protein expression status.

Results: In 5 of 10 lung cancer cases (50%) more than five EGFR signals per nucleus were identified, suggesting a possible gain in copy number. Interestingly, in four of 9 cases of AAH (44.4%) more than three EGFR signals per nucleus were noted, with scattered cells showing up to 6 signals per nucleus. In addition, in 6 of 12 cases (50%) of BAC, more than three EGFR signals per nucleus were noted. In the remaining cases 2 to 3 intranuclear dot-like peroxidase positive signals were present consistent with non-amplified gene.

Conclusions: Our study reveals an abnormal EGFR gene copy gain in several cases of atypical adenomatous hyperplasia (AAH), although early quantification relative to centromeric probes show balanced gain in copies of chromosome 7. The rate of EGFR gene copy abnormalities in AAH appears lower than in BAC and lower than in ACA. These findings suggest that EGFR gene copy abnormalities may be an early event in lung carcinogenesis and demonstrate that EGFR CISH may be an efficient method to assess the EGFR copy number abnormalities. Continued studies are needed to further define early molecular events in lung carcinogenesis.

1424 Epidermal Growth Factor Receptor in Non Small Cell Lung Cancer: Gene Copy Number and Mutational Study

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Background: Activating mutations of the *EGFR* gene were identified in tumors from patients with non small cell lung cancer (NSCLC) that responded to therapy with tyrosine kinase inhibitors (TKIs). More recently, survival has been reported to significantly increase among patients carrying a high number of copies of *EGFR* gene after treatment with erlotinib in comparison with those receiving placebo. Thus, there is a need to tailor individual treatments based on *EGFR* molecular profile.

Design: For this retrospective study, molecular characterization of *EGFR* was performed on formalin-fixed, paraffin-embedded specimens collected in the area of Barcelona. The series included 83 tumors obtained from patients with NSCLC (72 men and 11 women). Concerning histopathology, lesions were classified as adenocarcinoma (AC), bronchioloalveolar carcinoma (BAC), adenosquamous carcinoma, squamous cell carcinoma, or large cell undifferentiated carcinoma. Gene copy number was assessed by dual-color FISH using both a centromeric probe (CEP7) and a locus specific probe (EGFR LSI). Mutational study was conducted by PCR followed by bidirectional sequencing of exons 18 to 21.
Results: By FISH, 42 out of 76 evaluable cases (55%) were found to carry a high copy number of *EGFR* (ratio LSI/CEP7 ≥2 or gene copies ≥4 per tumoral nuclei). Of those, 9 (21%) were found to be amplified while 33 (79%) exhibited high polysomy (in ≥40% of tumoral nuclei). Out of 34 cases with a low copy number, 14 (41%) had disomy and the other 20 (59%) had trisomy or low polysomy (in <40% of tumoral nuclei). Neither histologic type (AC including BAC *versus* others) nor gender did significantly correlate with *EGFR* copy number. By sequencing, 3 mutations were detected out of 71 evaluable cases (4%) corresponding to large exon 19 deletions previously described (2 cases with delE746-A750 and 1 case with delL747-P753insS). Mutated cases were 2 AC and 1 BAC, all them from women who carried a high copy number of *EGFR* (2 with high polysomy and 1 with amplification).
Conclusions: In our series, FISH allowed identifying 55% of patients likely to benefit from TKIs therapies. Our results suggest that FISH is a better choice than sequencing to assist in discerning *EGFR* molecular profile of NSCLC. Further studies should be conducted to elucidate the role of *EGFR* alterations in the prognosis of NSCLC.

1425 Differential Epithelial Expression of *Sonic Hedge Hog* and *Foxfl1* in Interstial Pneumonias

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Background: Idiopathic pulmonary fibrosis results in simplification of lung parenchyma with end-stage cystic changes that resemble embryonic pre-alveolar lung . Its most common pattern, Usual Interstitial Pneumonia (UIP), has a poor prognosis. Non-specific interstitial pneumonias (NSIP) can be distinguished from UIP and have a better outcome. The fibrotic variant of NSIP (NSIP-F) can be difficult to distinguish from UIP histologically. The *sonic hedgehog* (*Shh*) pathway, that includes *Foxfl1, is* activated by cells of the proximal and distal pulmonary acinus, respectively, during embryonic branching morphogenesis.We examined the gene expression of *Shh* and *Foxfl1* by lining epithelium of end-stage lung cysts, in UIP and NSIP-F.
Design: The lungs of 13 patients and two normals were examined. The diagnosis of UIP was established histologically in 8 cases and NSIP-F in 5 cases. All cases showed areas of honeycomb lung. The mean long-axis diameter of honeycomb spaces was determined by image analysis. In situ hybridization was performed with digoxigenin-labeled RNA probes from human *Shh* and *Foxfl1*. Staining of cyst epithelium was scored from 0-+2 staining intensity by light microscopy. Data was analyzed by Graph Pad Prism 4 software.
Results: Subpleural honeycomb cystic spaces were identified in all cases of UIP and NSIP-F. The mean diameter of cysts in UIP was larger than in NSIP-F (0.39 ± 0.06 vs. 0.25 ± 0.05 cm, p=0.05). The cysts in UIP showed frequent branching and were lined by columnar or high cuboidal epithelium. The cysts in NSIP-F showed little branching and were lined by low cuboidal epithelium. *Shh* was strongly expressed in the cyst epithelial lining cells in UIP in all cases, and weakly (4/5) or strongly (1/5) expressed in the lining cells of NSIP (2.0 vs 0.9+0.04, p<0.05). *Foxfl1* was weakly expressed by lining cells in all cases of UIP, and strongly (4/5) or weakly (1/5) expressed in NSIP-F (p=0.08). Normal lung showed weak expression of *Shh* by bronchial epithelium and alveolar macrophages; there was minimal epithelial expression of *Foxfl1*.
Conclusions: UIP and NSIP-F differ in the architecture and size of end-stage cysts. High expression of *Shh* with concomitant low expression of *Foxfl1* in UIP suggests failure to activate *Foxfl1* and loss of the distal pulmonary acinus. The persistent expression of *Foxfl*1 in most cases of NSIP-F may indicate persistence of distal acinar progenitor cells and could account for its better functional outcome.

1426 Thymic Carcinoma Usually Lacks Activating Mutations in *KIT*

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Background: KIT (CD117) is a transmembrane tyrosine kinase receptor expressed in mast cells, germ cells and interstitial cells of Cajal. An homologous kinase, PDGFRA, is more widely expressed. Oncogenic mutations in the *KIT* gene are found in several tumor types including 78% of GI stromal tumors (GISTs). An additional 8% of GISTs have activating mutations in *PDGFRA*. The kinase inhibitor imatinib mesylate (*Gleevec*, Novartis Pharma) potently inhibits most mutant isoforms of KIT and PDGFRA, and has been a highly effective treatment for advanced GIST. In contrast, cancers that lack detectable *KIT* or *PDGFRA* mutations are largely unaffected by this medication. We have shown previously that thymic carcinomas, but not thymomas, express KIT (J Cancer Res Clin Oncol 2004; 130:222-224). Therefore, we have assessed the frequency of kinase gene mutations in these rare tumors.
Design: Samples of thymic carcinoma were collected with IRB approval. Analysis of *KIT* exons 9, 11, 13 and 17, as well as *PDGFRA* exons 12, 14 and 18, was performed using a combination of PCR amplification (GeneAmp PCR System 9700, Applied Biosystems) and denaturing HPLC (WAVE system, Transgenomic, Inc.), as previously described (Am J Pathol 2004, 164:305-313). KIT immunohistochemistry was performed on 5 micron unstained sections either treated with proteinase K for 5 minutes @ RT then covered with pre-diluted monoclonal anti-c-kit antibody (Oncogene) or rabbit antiserum (A4502, Dako), both followed by standard DAB detection. Appropriate controls were used throughout.
Results: 5 thymic carcinomas were available from 4 men and 1 woman, ranging in age from 23 to 65 with an average of 37.5 years. All 5 cases were strongly and diffusely KIT positive by immunohistochemistry. All 5 were wild-type for *KIT* exons 9, 11, 13 and 17. *PDGFRA* exons 12, 14 and 18 from 3 of the cases have been examined and were wild-type as well.
Conclusions: 5 thymic carcinomas expressing KIT by immunohistochemistry were found to lack activating mutations of *KIT*. As some KIT-positive GISTs harbor mutations in *PDGFRA* instead of *KIT*, we also examined this kinase gene. Again, no mutations were observed. These findings suggest that imatinib mesylate is unlikely to be useful in the treatment of most thymic carcinomas.

1427 cDNA Arrays Differentiate Benign from Malignant Mesothelial Cells in Effusions

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Background: Malignant mesothelioma (MM) is a highly aggressive tumor originating from the serosal cavities. The transformation of reactive mesothelial cells (RM) to MM involves the decades-long acquisition of multiple chromosomal aberrations. The phenotypic results of these alterations are still incompletely defined. The present study compared global gene expression in RM and MM in effusions.
Design: cDNA microarray analysis was performed on 9 MM and 4 RM effusions. Results were corroborated using quantitative reverse-transcription polymerase chain reaction (qRT-PCR), RT-PCR, flow cytometry and immunocytochemistry (ICC).
Results: Non-supervised clustering showed two branches, one with all RM specimens and one MM, the other with 8 MM specimens. Significance Analysis of Microarrays (SAM) identified 249 genes that were upregulated in MM compared with RM, including claudin 15, mesothelin, the α3 integrin subunit, vitronectin, cyclins D1 and G2, Stat1, EphA2, ephrin-B2, tissue inhibitor of metalloproteinases (TIMP-3), and MUC1. Ten genes were downregulated in MM cells, including desmin and the platelet-derived growth factor receptor α subunit (PDGFRα). Prediction Analysis for Microarrays (PAM) confirmed these data. qRT-PCR confirmed the findings regarding mesothelin and PDGFRα. ICC analysis of 40 MM and 50 RM confirmed the specific expression of MUC1 in MM and desmin in RM.
Conclusions: cDNA array analysis separates MM from RM and identifies a large number of genes that are upregulated in MM compared with RM. Corroboration of these results on protein level in larger series may aid in understanding molecular events that are related to tumor progression in MM and in identifying therapeutic targets for MM cells in effusions.

1428 Beta-Catenin and Estrogen-Receptor Beta Expression in Pulmonary Meningothelial-Like Nodules: Another Link in the "BROCN" Family of Lesions?

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Background: There is mounting evidence in the literature suggesting that beta-catenin (BC) pathway is one of the key events in hormone-related carcinogenesis. Recently, BC and estrogen-receptor beta (ER-beta) have been implicated in the development of biotin-rich optically clear nuclei (BROCNs), morular formations that occur in a variety of neoplasms, most of which are seen in women. Pulmonary meningothelial-like nodules (chemodectoma bodies) are incidental lesions of undetermined nature. We hypothesize here that MLNs are similar not only to meningiomas, but also BROCNs.
Design: The clinicopathologic features of 5 MLNs identified in the authors' files were investigated, and immunohistochemical stains for b-catenin and ER-beta were performed, then compared with results in 38 meningiomas.
Results: Four of 5 patients with MLNs were female, with a median age of 68. In 4 patients, MLN was detected incidentally in resections performed for other causes (1 primary carcinoma, 1 secondary carcinoma, 1 infectious, and 1 subpleural blebs) and in the 5th patient, it was the sole pathologic finding in a biopsy. In all 4 patients with available family history, there was a first-degree relative with cancer; however, none was known to have a history of FAP, which occurs in some BROCN cases. The morphologic features of MLNs were found to be remarkably similar to those of meningiomas and what has been recently documented about BROCNs: Tight whorls and clusters of plump spindle cells with a fair amount of cytoplasm, indistinct cell borders, and nuclear pseudoinclusions (optically clear nuclei). Cytoplasmic expression of b-catenin was present in all 5/5 (100%) of MLNs and 37/38 (97%) of meningiomas. ER-beta was also detected in all 5/5 MLNs (100%) and 36/38 (95%) of meningiomas.
Conclusions: MLNs have many clinical, morphologic and mechanistic similarities to "BROCNs" and meningiomas. They occur predominantly in females and express beta-catenin and ER-beta which suggests a hormone-related pathogenesis for these lesions, with activation of beta-catenin (wnt-signaling) pathway as one of the key events. Whether MLNs are indicators of a genetic predisposition to cancer, as all 4 cases with available family history had a first-degree relative with cancer, ought to be further analyzed. New insight into the pathogenesis of meningiomas and BROCNs may also be applicable to MLNs as well.

1429 Expression Pattern of Osteopontin and Correlation and Signalling Molecules in Lung Adenocarcinoma and Malignant Pleural Mesothelioma

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Background: Osteopontin (OPN) is a matricellular protein involved in tissue remodeling and cell-mediated immunity. High serum levels predict poor prognosis in NSCLC and other malignancies and, as we recently described, identify patients with malignant pleural mesothelioma (MM) vs. disease-free asbestos-exposed individuals. However, the tissue expression pattern of OPN in MM has not been detailed.

Furthermore, in vitro evidence links OPN to MMP's through NFK-beta (p65), and the EGFR signaling pathway. However, these associations have not been verified in vivo.
Design: We studied by immunohistochemistry (IHC) the OPN pattern of expression in adenocarcinoma (Aca) and MM and correlated it with morphological features, and presence of p65, activated EGFR signaling pathway molecules p-AKT, p-ERK and p-Stat3 and metalloproteinases MMP1, MMP2, MMP9. Ninety-one Aca were classified in low grade (bronchioloalveolar+well-differentiated, n=46) and high grade (moderately+poorly-differentiated, n=45). MM included 20 epithelioid, 16 biphasic and 2 sarcomatoid. The proliferative rate was measured by Ki-67 stain. OPN and all other markers were scored by multiplying percentage of stain by intensity, each expressed in a scale of 0 to 3. All results were grouped in low (0-4) versus high (5-9) score.
Results: In Aca high OPN score showed a significant statistical association with lower vs high grade. In Aca a positive correlation existed between p65 and MMP-9 and between p65 and pAKT. OPN expression was almost universal in MM: 36/38 and showed no correlation with any of the molecules studied.
Conclusions: Widespread tissue expression of OPN in MM supports its demonstrated value as a serum marker discriminating between patients with MM and disease-free asbestos-exposed individuals. In Aca, but not MM, high OPN expression is correlated with low tumor grade, and p65 and MMP-9 expression, suggesting a functional axis regulating MMP-9 expression via a p65-mediated pathway. The expression pattern of OPN expression differs in Aca and MM, implying a different biological role of OPN in these malignancies.

1430 Nuclear Maspin Predicts Favorable Morphological and Molecular Features of Lung Adenocarcinoma and Pleural Malignant Mesothelioma

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Background: Maspin (Ma) is a Mammary Serine Protease Inhibitor tumor suppressor gene. It is frequently altered in cancer, yet it is controversial whether its nuclear (N), versus cytoplasmic (C) localization affects its biology. Particularly, it is unknown whether its subcellular localization is a target of carcinogenesis in the lung and whether it correlates with the pathological features of Non Small Cell Lung Carcinoma (NSCLC) and malignant mesothelioma (MM).
Design: To test whether transformation affects levels and subcellular localization of Ma in vitro, we induced the malignant phenotype by chemical treatment in the bronchial epithelial cell line BEAS2-B, and studied Ma by Western blot in whole cell lysates, N and C subcellular fractions, and confocal microscopy. To study the relevance of Ma localization in vivo, we then analyzed by immunohistochemistry (IHC)123 NSCLC and 66 MM and correlated Ma N vs N+C expression with tumor's histotype, grade, proliferative rate (Ki-67), vEGF and p53 expression. Ki-67 was scored as % of positive tumor cells,VEGF by a score obtained multiplying intensity by percentage of positivity, each in a scale of 1 to 3. P53 was scored positive when present in ≥10% tumor cells.
Results: Chemical transformation of bronchial epithelial cells selectively decreases N Ma protein levels. By IHC Ma is expressed in 72/77 Adenocarcinoma (Aca): 50 N, 22 N+C; 46/46 Squamous Cell Carcinoma (SqCCa): 37/46 N+C, 2 N, 7 C; 30/66 MM: N 12/30, N+C 18. In Aca, N vs. N+C Ma is associated with lower histological grade (p.001), lower proliferative rate (p=.010), and absent p53 stain (p 0.01). In Aca and MM, N Ma correlates with a low (≤4/9) vEGF score (p 0.025).
Conclusions: An in vitro model shows that transformation is linked to reduction of N Ma. In vivo, N Ma selects Aca and MM with favorable morphological and molecular features, including reduced angiogenesis. The expression pattern of Ma and likely, its biological significance differ between Aca and SqCCa. However, our overall data support the hypothesis that the tumor suppressor activity of Ma is linked to its N expression, and thus suggest an intriguing possible mechanism of tumor progression.

1431 Lymphohistiocytoid Variant of Mesothelioma: A Series of 21 Cases

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Background: Lymphohistiocytoid mesothelioma may be misdiagnosed as malignant lymphoma and other neoplasias eliciting florid lymphoid responses. We report on the clinico-pathological and immunohistochemical features, EBV status and survival data of 21 cases.
Design: 21 MMHL cases were retrieved from the Mesopath group files over a study period of 1998-2004. Clinical, demographic data and survival from date of diagnosis were recorded using a structured questionnaire. Cases fulfilling the following morphological criteria were included: diffuse sheets of histiocytoid cells with prominent lymphoid infiltrate. All cases exhibited typical mesothelial immunophenotype an additional immunohistochemical panel of lymphoid markers included CD45, CD3, CD4, CD8, CD20, CD56, CD30,CD68, S100 , TdT. EBV status was evaluated using LMP1 in conjunction with EBER-1 RNA ISH. Occupational histories were evaluated by a group of epidemiologists.
Results: There were 21 patients with an average age of 69.8 yrs (range 50 to 82 years) including 14 males and 7 females. Clinical symptomatology included chest pain, fatigue and weight loss. Patients presented with hemorrhagic or lymphocytic pleural effusions (16), diffuse pleural thickening with or without nodularities (15), a pleural based nodule (2) and hyaline fibrous plaques (4). Fifteen cases died of disease with a median survival of 8.2 months while 6 are alive with disease at 11-34 months. Eleven patients had a previous history of heavy asbestos exposure. Patients had had palliative surgery (7) and pleurodesis alone or in association with radiotherapy (10). Chemotherapy (11) was done for the others. Histological findings consisted of a diffuse proliferation of large histiocytoid, discohesive cells with vesicular pleomorphic nuclei and rounded prominent nucleoli. Rare "Reed Sternberg- like cells " were identified. All neoplastic cells were strongly positive for AE1/AE3 and calretinin. None of the large histiocytic cells demonstrated positivity for CD30, CD45, or S100 protein. The immunophenotype of the lymphoid component showed a heavy T cell cytotoxic population (CD3+, CD8+). T helper cells (CD4+) and B cells (CD20+) were scarce. No immature T cells (TdT+) were seen. EBV (LMP1 and EBER 1) status was negative in all cases.
Conclusions: MMLH is a rare variant of mesothelioma showing a heavy T cell cytotoxic population. This tumor does not appear to be related to EBV infection.

1432 Pleuropulmonary Involvement in Pseudomyxoma Peritonei

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Background: Pseudomyxoma peritonei (PP) is an uncommon clinical condition associated with infradiaphragmatic dissemination of mucinous neoplasms of the appendix. Involvement of the lung or pleura is exceedingly rare. We report the clinical and morphologic features of 4 patients with supradiaphragmatic disease following therapy for PP.
Design: We recently reviewed 101 patients treated uniformly at our institution with intraperitoneal hyperthermic chemotherapy for PP of appendiceal origin. Four had pleuropulmonary involvement during their clinical courses. They comprise the subjects of this in-depth review of their cytology, histology, and clinical records.
Results: Our subjects comprised 3 men and 1 woman who were 38, 43, 61, and 41 years old when first diagnosed with PP. Two had low grade tumor histology in the peritoneum; both were composed of variably proliferative, bland-appearing mucinous neoplastic epithelium, arising from low grade appendical mucinous neoplasms. Both developed one or more pulmonary parenchymal metastases of low histologic grade (LHG), some of which were resected. In the woman with PP of LHG, her sole metastasis was composed of cytologically benign tumor cells (TC) with low nuclear-to-cytoplasmic ratios (N/C). Characteristically, the TC were arranged in well polarized strips at the interface between bands of dense fibrous connective tissue and voluminous extracellular mucin (ECM). Sclerotic tissue formed a partial boundary between tumor and pulmonary parenchyma. In the other patient with PP of LHG, the lung masses were mostly composed of ECM. Although well differentiated with low N/Cs, they showed focal cribriform architecture, increased nuclear atypia, and elevated cellularity. The lack of pleural involvement militates against transdiaphragmatic tumor extension. The 2 men with PP of high histologic grade developed right-sided pleural effusions, 7 and 17 months after their initial diagnosis of PP, respectively. The background of the cytologic smears contained wispy mucin. High grade adenocarcinoma cells were present singly, in small clusters, and large spheres. No thoracic nodal metastases were identified in any patient.
Conclusions: Although most uncommon, mucinous neoplasms from PP may involve the thorax. We believe that our data further blurs the distinction between adenomucinosis and well-differentiated mucinous carcinoma, supporting our notion that all forms of PP are clinically malignant.

1433 Cytokine Polymorphisms and Lung Cancer Risk

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Background: Lung cancer is the leading cause of cancer deaths worldwide. Tobacco smoking is the main risk factor for lung cancer, however the inflammatory/immune system may play a role in lung carcinogenesis. Cigarette smoke stimulates airway epithelial cells to release proinflammatory cytokines, which trigger a cascade of inflammatory reactions through the induction of inflammation-related substances. Cytokine levels have recently been associated with the development of lung cancer, and polymorphisms of cytokine genes may be involved in regulating the inflammatory response and consequently lung cancer risk.
Design: The purpose of this study was to assess the relationship between single nucleotide polymorphisms (SNPs) of five cytokine genes involved in the inflammatory cascade (TNF-α, TGF-β, IL-10, IL-6, and IFN-γ) and the development of lung cancer. Genotyping was performed on the peripheral blood of 99 lung cancer patients with significant smoking histories and 99 matched controls, using a commercially available kit for all 5 cytokines.
Results: Using multiple logistic regression modeling, most of the SNPs evaluated were not associated with a significant difference in lung cancer risk. However, the GCC/ACC genotype of IL-10 (SNPs –1082, -819, -592) showed a significant increase in risk of lung cancer in the whole patient population when compared to controls (odds ratio (OR)=2.69, 95% confidence interval (CI)=1.14-6.30). Women were at particularly higher risk of lung cancer with an OR=8.21 (95% CI=1.83-36.86) for the GCC/ACC genotype. In addition, the T/A genotype for the +874 SNP of IFN-γ (OR=2.23, 95% CI=0.92-5.41) was associated with a borderline significant increased risk of lung cancer.
Conclusions: Individuals, and especially women, with the genotype associated with intermediate/low expression levels of IL-10, and both genders with the intermediate expression levels of IFN-γ may be at increased risk of lung cancer. Although the sample size in this study is limited, our data suggest that these polymorphisms may be associated with risk of lung cancer, through an inflammatory response mechanism. To our knowledge, this is the first study to suggest an association of IL-10 and IFN-γ gene polymorphisms with lung cancer risk.

1434 Loss of Expression of MLH1 and BRCA2 Is Associated with Progression of Lung Adenocarcinoma

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Background: In lung carcinogenesis, studies have shown a stepwise accumulation of genetic abnormalities which correlate with increasing morphologic changes. Within the adenocarcinoma sequence, atypical adenomatous hyperplasia (AAH) is considered

a preneoplastic lesion, and a possible precursor of bronchioloalveolar carcinoma (BAC). There is good evidence that AAH may progress from low to high grade to BAC. Invasion then develops within BAC and invasive carcinoma evolves. The molecular events associated with this progression are not well understood. In this study, we examined the role of DNA mismatch repair (MMR) genes (MLH1, MSH2) and BRCA1 and 2 (which play roles in DNA recombination and/or repair) in the progression of lung adenocarcinomas by examining the rates of expression of these proteins using immunohistochemical methods.

Design: In this study, we reviewed surgical specimens from 67 cases of AAH and lung adenocarcinoma treated at New York University Medical Center from 1992-2005. All cases were reviewed and classified according to the 2004 WHO Classification system, and were subdivided into AAH (7 cases), BAC (15 cases), BAC with invasion <5 mm in diameter (11 cases), BAC with invasion >5 mm (15 cases), and purely invasive carcinoma (19 cases). A tissue microarray of cores from paraffin embedded tissue samples was constructed and analyzed for MLH1, MSH2, BRCA1, and BRCA2 using immunohistochemical procedures on an automated immunostainer. Tumor cells showing any degree of nuclear staining were considered positive.

Results: MLH1 and BRCA2 showed loss of expression in tumor cells starting from the BAC<5 mm group. MSH2 and BRCA1 showed no loss of expression in any of the groups. See table for more complete results.

Conclusions: In prior studies it has been shown that there is a statistically significant difference in 5-year survival between the BAC/BAC<5mm group and BAC>5mm/purely invasive carcinoma group, with the latter groups showing significantly higher mortality rate. In our study, loss of expression of MLH1 and BRCA2 was seen only in the latter groups. These results suggest that loss of expression of MLH1 and BRCA2 is associated with tumor aggressiveness, while MSH2 and BRCA1 seem to have no role in tumor progression.

Immunostaining Patterns of DNA Repair Proteins

	MLH1	MSH2	BRCA1	BRCA2
AAH	100%	100%	100%	100%
BAC	100%	100%	100%	80%
BAC+<5mm	73%	90%	100%	100%
BAC+>5mm	73%	94%	100%	73%
Invasive	58%	100%	95%	53%

1435 Myoepithelial and Salivary Duct Differentiation Markers in Non-Small Cell Lung Cancer

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Background: While most non-small cell lung cancers (NSCLCs) arise from the surface epithelium of conducting airways or alveoli, occasional malignancies originate in the bronchial submucosal glands. Histologic features may indicate a salivary gland-type neoplasm, but some cases may be ambiguous, leading to an interpretation of NSCLC. This study was undertaken to determine the expression of myoepithelial and salivary duct differentiation markers in NSCLC.

Design: High-throughput tissue microarrays containing 340 NSCLCs with more than five years of clinical follow-up were studied. Immunohistochemical staining for calponin (1:1000; DakoCytomation), caldesmon (1:500; DakoCytomation), p63 (1:100; Novocastra), and gross cystic disease fluid protein-15 (GCDFP) (1:500; Signet) was performed. A composite score of 0-3 was assigned to each tumor, reflecting mean staining intensity [graded as 0 (negative), 1+ (weak), 2+(moderate), or 3+(strong)] and the mean percentage of tumor cells staining, calculated from the triplicate punch samples of each tumor. Clinical information including patient survival, tumor stage, patient age at diagnosis, and smoking history was obtained. Correlation of expression of markers and clinicopathologic variables was statistically analyzed using Spearman rank correlation and Kaplan-Meier analysis.

Results: P63 expression was characteristic of most squamous cell carcinomas (83.7%), but less common in large cell carcinomas (25.0%) and infrequent in adenocarcinomas (5.3%). Expression of calponin and caldesmon was rare, with only 5 positive cases in this cohort (n=340). GCDFP was expressed by small numbers of adenocarcinomas (5.3%), large cell carcinomas (3.6%), and squamous cell carcinomas (2.3%). In early stage cases, Kaplan-Meier analysis showed a trend (p=0.06) towards poorer 5-year survival in calponin-expressing tumors (although only 2 cases were calponin-positive). There was no relationship between expression of p63, caldesmon, or GCDFP, and five-year survival.

Conclusions: Although p63 is expressed by most squamous cell carcinomas, expression of other markers of myoepithelial differentiation is very uncommon. GCDFP is found in a small minority of adenocarcinomas, raising the question of salivary duct differentiation in these neoplasms.

1436 Pulmonary Neuroendocrine Tumors Can Express Markers of Myoepithelial and Salivary Duct Differentiation

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Background: Pulmonary neuroendocrine tumors (NETs) and salivary gland-type neoplasms usually originate in the large airways and can demonstrate overlapping histologic features in small samples. Immunohistochemistry is often employed in the evaluation of these tumors. Although expression of neuroendocrine substances by NETs has been studied extensively, expression of markers of salivary gland cell populations has received relatively little investigation.

Design: Three tissue microarrays were constructed from 111 pulmonary NETs: 54 carcinoid tumors (CARs), 11 large cell neuroendocrine carcinomas (LCNECs), and 46 small cell carcinomas (SCCs). Triplicate punch samples of formalin-fixed paraffin-embedded tissue from each tumor were included. Immunohistochemistry was performed for myoepithelial markers including calponin (1:1000; DakoCytomation), caldesmon (1:500; DakoCytomation) and p63 (1:100; Novocastra). A marker of salivary duct differentiation, gross cystic disease fluid protein-15 (GCDFP)(1:500; Signet), was also evaluated. For each stain, a score of 0-3 was assigned to each tumor based on the mean percentage of stained neoplastic cells and the mean staining intensity of tumor cells (0=no staining, 1=weak, 2=moderate, 3=strong). Spearman rank correlation was used to assess the relationships between marker expression and the presence or absence of metastasis.

Results: Calponin was most commonly expressed by the NETs: CARs 13.0%, LCNECs 27.3%, SCCs 19.6%. Caldesmon expression was less frequent: CARs 7.4%, LCNECs 9.1%, SCCs 2.2%. No p63 expression was noted in LCNEC, but 5.6% of CARs and 6.5% of SCCs showed expression of this marker. GCDFP stained 3.7% of CARs, 18.2% of LCNECs, and 2.2% of SCCs. There was no significant correlation between expression of calponin, caldesmon, p63, or GCDFP, and metastasis.

Conclusions: Immunohistochemical staining with markers of myoepithelial or salivary duct differentiation can be observed in a significant minority of pulmonary NETs. This may be a potential source of confusion in the differential diagnosis with primary salivary gland-type tumors of the lung, particularly in small samples.

1437 Molecular Alterations in Atypical Adenomatous Hyperplasia Occurring in Benign and Cancer-Bearing Lungs

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Background: Atypical adenomatous hyperplasia (AAH) is most frequently an incidental finding in lungs resected for primary carcinoma, but can also be seen in lungs that harbor benign processes. There is good morphologic evidence that AAH may progress to bronchioloalveolar carcinoma and/or invasive adenocarcinoma (AC). Several genetic abnormalities have been reported in AAH associated with AC, but little is known about genetic changes in AAH associated with benign lesions. The aim of this study is to compare the molecular characteristics of AAH present in benign conditions to those co-existing with carcinoma to assess AAH as a possible premalignant lesion.

Design: Eight cases of AAH from resected non-neoplastic lungs and 11 cases from lungs with primary AC were retrieved from the paraffin-block archives of the University of Pittsburgh Medical Center. Sixteen of 19 patients had a documented history of cigarette smoking. Loss of heterozygosity (LOH) was analyzed on microdissected normal and lesional tissue by PCR using 21 fluorescently labeled polymorphic microsatellite markers located on 3p, 5p, 7p, 9p, 10q, and 17p. The frequency of allelic loss (FAL) was calculated by dividing the number of alleles lost by the number of informative loci. Direct DNA sequencing for K-ras mutations was also performed

Results: AAH associated with AC showed LOH in 9% of cases (1/11) at 3p, 7p and 10q, but no LOH at 9p. AAH from non-neoplastic lungs showed LOH in 50% of informative cases (4/8) at 3p, 12.5% (1/8) at 7p, 9p and 10q. Neither group showed LOH at 5p or 17p. Although there were some differences in FAL between the two groups, these differences were not statistically significant. K-ras mutations were not identified in either group.

Conclusions: Loss of heterozygosity of chromosomal loci frequently affected in lung carcinogenesis appears to be an uncommon event in AAH, with or without co-existing AC. Therefore, AAH may represent a smoking induced low-grade neoplastic lesion that may be a precursor lesion of only a subset of invasive lung AC.

1438 Loss of E-Cadherin Immunoexpression Is Associated with Poor Survival in Non-Small Cell Lung Cancer

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Background: E- Cadherin is a transmembrane glycoprotein involved in intercellular adhesion. Loss of expression of E-cadherin is associated with disease progression, recurrence and poor prognosis in urothelal, gastric, and pancreatic cancers. Animal models suggest that E-cadherin may serve as both a tumor suppressor and an invasion suppressor in lobular carcinoma of breast . We examined 147 surgically excised non-small cell lung cancers (NSCLC) for E-cadherin expression and determined correlation with survival.

Design: Tissue Microarrays prepared from 147 NSCLC were immunostained with E-Cadherin antibody (1:25; Cell Signaling, Beverly, MA) using standard avidin-biotin technique. Three punches were used for each case, each punch scored for percentage of cells stained and intensity of staining using a scale of 0-100 and 1-3 respectively, and each value averaged at the end of scoring. Information about survival was obtained from patients' medical records. Data were analyzed using Spearman rank correlation and Kaplan-Meier analysis.

Results: Strong E-cadherin staining was seen in 83 (56.5%) tumors, and weaker staining in 64 (43.5%) tumors. A significant inverse correlation was noted between higher tumor grade and E-cadherin staining (p=0.007). For all subjects, strong E-cadherin expression was associated with significantly better survival than weaker expression(p=0.0243). While in the early stage squamous carcinoma (n=27), stronger E-cadherin expression was associated with better survival (p=0.0061), E-Cadherin expression showed no relationship to survival (p=0.483) for early stage adenocarcinoma (n=55).

Conclusions: E-cadherin may be an important factor influencing prognosis and survival in patients with NSCLC. E-Cadherin loss may be an important step in the progression of early stage squamous cell carcinoma of lung.

1439 Primary Pulmonary and Mediastinal Synovial Sarcoma (PPMSS): Single Institution Study of 43 Cases

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Background: Soft tissue synovial sarcoma (STSS) occurs in extremities of young patients and contains notable calcification and scattered mast cells. Recent series discuss synovial sarcoma (SS) as an aggressive primary pulmonary/mediastinal neoplasm with features similar to STSS. We reviewed our experience with PPMSS to identify tumor-specific histologic features that narrow the differential diagnosis to guide molecular studies, and to recognize associated pulmonary findings.

Design: 99 thoracic cases diagnosed as SS or sarcoma NOS were retrieved from our files. Morphology, immunohistochemistry (IHC), and molecular details were reviewed. One lung metastasis and 55 tumors lacking SS diagnostic criteria [morphologic features, epithelial IHC, t(X;18)] were excluded.

Results: 43 PPMSS included 20 males, 17 females, 6 unknown sex. Age: 10-84 years, mean 43. Patients had smoking history (8/14), recurrent pulmonary infection/inflammation (5/43), blebs/spontaneous pneumothorax (3/43), antecedent chest trauma (2/43), and asbestos exposure (2/43). Tumor size: 0.6-17 cm, mean 6.4. Tumor location: pleura (17/43), left lung (13/43), right lung (9/43), mediastinum (4/43). PPMSS were well demarcated with fibrous capsules abutting bronchial cartilage or pleura. 29 were monophasic, 5 biphasic, 9 poorly differentiated. Tumors had pink collagenous stroma (43/43), inconspicuous mast cells (43/43), HPC-like features (36/43), necrosis (29/43), myxoid change (23/43), epithelioid morphology (19/43), and calcification (6/43). Mitoses: 1-95/10 hpf, mean 13. Positive IHC: pankeratin (29/41), CK7 (23/33), EMA (22/40), Bcl2 (16/16), calretinin (13/16), CD99 (11/14), CD56 (9/9), S-100 (8/39), Cam5.2 (7/28), and CD34 (0/33). 23/43 had entrapped TTF-1 positive epithelium. Positive t(X;18): 22/23 (15 SYT/SSX1, 6 SYT/SSX2, 1 unknown, 20 pending). Associated histologic lung findings included central location (15/26) and respiratory bronchiolitis/emphysema (22/43).

Conclusions: Most PPMSS are monophasic, and occur in central or pleural locations. Entrapped lung epithelium is common and should not be misinterpreted as biphasic SS. Two-thirds of patients had evidence of antecedent lung injury that may have contributed to tumor development. In contrast to STSS, PPMSS occurs in older patients with less calcification and mast cells. Attention to morphology and epithelial IHC avoids misclassification of PPMSS, and affords prudent use of confirmatory t(X;18) studies.

1440 Immunohistochemical Analysis of Glutathione S-Transferase alpha and Insulin Growth Factor Binding Protein-3 in Primary Pulmonary Adenocarcinoma

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Background: Clear cell carcinomas are relatively common tumors arising in many locations, such as the kidney, female genital tract, adrenal cortex, and lung. The distinction between clear cell renal cell carcinoma (RCC) and lung adenocarcinoma may present a diagnostic dilemma. Upregulation of both GST-α and IGFBP-3 has been demonstrated previously in primary clear cell renal cell carcinoma. We hypothesize that immunohistochemical analysis with GST-α and IGFBP-3 antibodies may be helpful in the differentiation between primary lung adenocarcinoma and metastatic clear cell RCC.

Design: A tissue microarray was constructed from forty-three (43) primary lung adenocarcinomas and exposed to both GST-α and IGFBP-3 antibodies respectively. In the GST-α group, we used seven (7) cases of metastatic clear cell RCC from various sites, including three cases from the lung, for comparison. Only RCC metastatic to the lung was analyzed for IGFBP-3. The immunoreactive intensity of each case was graded semiquantitatively as follows: negative, 0; weakly positive, 1+; moderately positive, 2+; or strongly positive, 3+. The distribution of positive cells was divided into focal (<30%) and diffuse (>30%).

Results: In the GST-α group, the great majority of lung adenocarcinomas (36/43, 83.7%, average intensity = 0.7) were focal or negative. Seven (7) cases stained diffusely (7/43, 16.3%, average intensity = 2.14). The metastatic clear cell RCC's demonstrated marked strong, diffuse positivity in the majority of cases (5/7, 71.4%, average intensity = 2.8). Two of the three cases metastasic to the lung stained intensely and diffusely. Both of the GST-α negative metastatic RCC cases were high grade tumors (Fuhrman grade 4) with marked pleomorphism and anaplasia. In the IGFBP-3 group, none (0/43) of the primary lung adenocarcinomas showed any positivity, and all (3/3) of the clear cell RCC lung metastases stained strongly and diffusely, including the high grade lung metastasis.

Conclusions: Our data demonstrate that, in contrast to metastatic clear cell RCC, primary lung adenocarcinomas are predominantly negative for GST-α and IGFBP-3. Strong GST-α expression is seen in low grade metastatic clear cell RCC with loss of GST-α in high grade tumors, consistent with previous reports. However, even in high grade metastatic RCC, IGFBP-3 maintains a strong, diffuse immunohistochemical staining profile. Additional cases will be identified and analyzed to further substantiate the findings of this study.

1441 Hypertensive Vasculopathy in Pulmonary Sequestration

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Background: Pulmonary sequestrations are defined by the lack of a connection with the upper tracheobronchial tree. They are divided into extralobar (ELS) and intralobar (ILS) types, both usually having an anomalous vascular supply from the systemic circulation, usually a direct branch of the thoracic or abdominal aorta. Although described in rare case reports, the vascular changes have not been studied systematically.

Design: Fourteen patients with pulmonary sequestration who underwent surgery from 1993-2005 were identified from our pathology database. Data analyzed included age, sex, type of sequestration, and histologic findings, including vascular changes (Heath and Edwards grading scheme).

Results: There were nine females and five males, age 5 days to 62 years. A summary of the findings are presented in the table.

Age/Sex	Sequestration Type	Location	Grade
2da F	ELS	Right lung	1
5da F	ELS	Right lung	0
6da F	ELS	Left lung	0
6da M	ELS	LUL	0
7da F	ELS	Left lung	0
2mo F	ILS	Right lung	2
3mo M	ELS	Right lung	1
3mo F	ELS	RLL	4
17mo M	ELS	Left lung	4
13y F	ILS	LLL	2
26y F	ILS	LLL	2
33y F	ILS	RLL	6
42y M	ILS	LLL	3
62y M	ILS	RLL	5

One case had necrotizing vasculitis. This was a 33 year old African-American female, with a history of right lower lobe pneumonia 6-7 years ago. Histologic exam revealed multiple dilated cystic air spaces. In addition, several medium-sized arteries showed necrotizing vasculitis with fibrinoid necrosis of the wall and a mixed inflammatory infiltrate, composed of neutrophils and lymphocytes, extending throughout the thickness of the wall. Follow up of this case revealed no clinical or serologic evidence of systemic/connective tissue disease.

Conclusions: A range of pulmonary hypertensive changes was seen in cases of both extralobar and intralobar sequestration. In the extralobar sequestration cases, there was a trend of worsening grade as the age of the patients increased. Previously unreported in the literature was one case of necrotizing vasculitis (grade 6 hypertensive change) in a patient with intralobar sequestration. The range of vascular changes seen in these cases seems to be similar to the way that pulmonary arteries react to pulmonary hypertension, including intimal proliferation and fibrosis, medial hypertrophy, plexiform lesions, and rarely, necrotizing vasculitis. The systemic artery supplying the sequestration is likely transmitting the systemic blood pressure to pulmonary vessels structurally ill-adapted to manage this pressure normally.

1442 Intratumoral, Peritumoral, and Adjacent Lung Lymphatic Vessel Density (LVD) and Lymph Node (LN) Metastasis in Non-Small Cell Lung Carcinoma (NSCLC)

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Background: Angiogenesis is an essential component of the metastatic pathway, and for many tumors, the vascular density has been shown to be an indicator of metastatic potential. The role of tumor lymphangiogenesis in relation to the risk of LN metastasis and to clinical outcome in patients with NSCLC is unclear at this time. To better define this relationship, we used D2-40, a novel monoclonal antibody that specifically reacts with lymphatic endothelium, to immunostain a series of NSCLCs and correlated this with LN status.

Design: We examined the intratumoral, peritumoral, and adjacent uninvolved lung LVD in 87 cases of NSCLC (25 squamous cell carcinomas, 51 adenocarcinomas, and 11 large cell carcinomas) using D2-40 IHC on formalin-fixed, paraffin-embedded tumors arranged on tissue microarrays (TMA). TMA for each tumor included a 1mm punch from the center of the tumor, the invasive edge of the tumor, and the adjacent uninvolved lung. LVD was counted as the number of lymphatic vessels counted per 1mm punch. LVD was correlated with LN status at the time of primary tumor resection.

Results: For all NSCLC, intratumoral LVD was significantly lower when compared to peritumoral LVD (Mann-Whitney test; $p<0.0001$) and uninvolved adjacent lung LVD ($p=0.0002$). Intra- and peritumoral LVD showed no correlation with LN status. For all NSCLC taken as a whole, there was a significantly higher LVD in the adjacent uninvolved lung tissue in patients with LN metastasis than in patients without LN metastasis ($p=0.0072$). Adjacent lung median LVD in patients without LN involvement was 1 (range 0-11), while in patients with LN metastasis it was 3 (range 0-23).

Conclusions: Involvement of regional LN is an important component in staging and ultimately the prognosis of patients with NSCLC. Although small studies in uterine cervical squamous cell carcinoma (SCC), head and neck SCC, and breast cancer have shown an association of high peritumoral LVD and nodal metastasis, this was not the case in our analysis. Our results suggest that intratumoral and peritumoral LVD as assessed by D2-40 immunostaining is not useful as a marker for LN metastasis in NSCLC. Interestingly, we found a correlation between the underlying normal lung LVD and LN status suggesting the possibility that an inherent high pulmonary LVD may predispose to LN metastasis rather than the type of tumor or location of lymphatics in specific relation to the tumor.

1443 EGFR in Malignant Mesothelioma of the Pleura: Analysis of Protein Expression, Gene Copy Number, and Transcript Levels

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Background: Alterations of the epidermal growth factor receptor (EGFR) gene and its protein have been reported in a variety of cancers including lung adenocarcinomas. EGFR status is increasingly important as EGFR has become a therapeutic target for humanized monoclonal antibodies and small molecule inhibitors. While pleural mesotheliomas are known to express EGFR, there have been few studies examining the correlation of EGFR expression with gene copy number and transcript levels in this cancer.

Design: We studied 46 cases of primary pleural malignant mesothelioma using tissue microarrays (TMA). EGFR protein expression and gene copy number were respectively detected by immunohistochemistry (IHC) (Clone 31G7, Zymed) and chromogenic in situ hybridization (CISH) (Spotlight EGFR CISH kit, Zymed) on consecutive sections.

The IHC staining was scored using a 4-tier scoring system. CISH signals were counted in at least 40 tumor nuclei. EGFR mRNA transcript levels, as derived by Affymetrix MAS5.0 from U133A chip hybridization data (probe set 201983_s_at), were available in 36 cases.
Results: By IHC, 5 (11%) cases showed 1+, 26 (56%) 2+, and 11 (24%) 3+ positivity. Four (9%) cases were negative by EGFR IHC. By CISH, the average signal number per nucleus was less than 2 (1.35-1.87) (as expected in tissue sections). An average signal number greater than 2 (2.01 - 2.95) was seen in 5 cases with 2+ staining and in 1 case with 3+ staining. The latter case also had the second highest EGFR transcript levels (the highest being from another sample from the same patient). EGFR mRNA transcript levels were significantly higher in IHC3+ cases compared to 0-1+ cases (p=0.017) or 0-2+ cases (p=0.046).
Conclusions: The majority of primary pleural mesotheliomas are positive for EGFR by IHC. Strong IHC staining for EGFR in pleural mesotheliomas correlates with mRNA levels but is not associated with gene copy number changes, with rare exceptions. The low level gene copy number increases observed are more consistent with polysomy of chromosome 7 rather than genuine EGFR gene amplification.

1444 Tissue Microarray and Immunohistochemical Analysis of Neuroendocrine Differentiation in Non-Small Cell Lung Carcinoma (NSCC)

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Background: The significance of neuroendocrine (NE) differentiation in lung carcinoma and the optimal means of identifying such differentiation are controversial. This study assesses the frequency and significance of expression of the most commonly used NE markers, synaptophysin (SNP) and Chromogranin (Ch) using tissue microarray (TMA) and immunohistochemistry technology.
Design: 593 NSCC were identified from the archives of St. Paul's Hospital, Vancouver, British Columbia. TMA blocks were made using duplicate 0.6-mm-diameter tissue cores from each marked paraffin block. Immunostaining was scored as positive if 5% or more of tumour cells showed immunoreactivity, and negative otherwise. The processed score data were analysed with the SPSS statistical software package and correlation analysis was performed with the bivariate 2-tailed Pearson Chi-square test.
Results: Hematoxylin-eosin stained sections of were reviewed and subclassified as follows: 245 adenocarcinoma (ACA), 262 squamous cell carcinoma (SQCA), 66 large cell carcinoma and 20 other (carcinosarcoma, giant cell carcinoma). NSCC showed reactivity for Ch only in 0.4% of cases (2/529), positivity for SNP alone in 7.6% of cases (40/526), while only 0.2% of cases (1/504) showed immunoreactivity for both markers. ACA were more frequently positive for SNP or Ch (23/212, 10.8%), compared to SQCA (11/232, 4.7%), otherwise there was no correlation between immunoreactivity and tumour morphology.
Conclusions: 1. SNP stains a significant minority of NSCC, while Ch immunoreactivity is very uncommon. Positivity for both NE markers is rare. 2. There is no significant correlation between positivity for SNP and Ch (p=0.25). 3. ACA are more likely to show NE differentiation as compared to SQCA (p=0.02).

1445 Identification of Aggressive Low Stage Non-Small Cell Lung Carcinomas by Cell Based, Multiparameter Molecular Analysis

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Background: The outcome of low stage non-small cell carcinomas (NSCLC) varies (Stage I NSCLC, 60% 5 year survival; Stage II, 40% 5 year survival). With laser scanning cytometry (LSC), we attempted to identify a subset of low stage NSCLC having an aggressive course that could benefit from chemotherapy.
Design: LSC was performed on fixed single cell suspensions from 48 primary NSCLC patients with low stage (25 stage IA, 23 IB, 1 IIA, and 10 IIB) lung cancers having a median follow-up of at least six months. Histologic types were 24 adenocarcinomas, 19 squamous cell carcinomas, 4 large cell carcinomas, 1 adenosquamous cell carcinoma. LSC involved four quantitative correlated fluorescence measurements per cell consisting of DNA cell content, Her2/neu, EGF receptor and VEGF on each of several hundred to several thousand) cells per tumor sample. Repetitive sequential analysis was performed with the first analysis identifying cells with EGFR > 30K molecules (mol.)/cell, Her2/neu > 1000K mol./cell , and aneuploidy > 3% in the same cells. 2nd analysis identified cells with Her2/neu > 1000K mol./cell, VEGF > 1000K mol./cell and aneuploidy > 3% in the same cells. Analysis #3 identified cells negative for EGFR and VEGF and aneuploidy.
Results: There were 13 recurrences (R) in 48 patients. 10 of these 13 R were identified using repetitive analysis with the three combinations of molecular abnormalities that were present in the same cells. 4/6 tumors with aneuploidy, Her2/neu overexpression and EGF receptor overexpression recurred. 3/4 with Her2/neu overexpression and VEGF expression recurred. 3/4 aneuploidy that underexpressed both EGF receptor and VEGF recurred.
Conclusions: Repetitive LSC identified 10/13 (43%) aggressive low stage NSCLC. In Stage IA, NSCLC, there were 4 R/21 patients; IB, 7 R/18 patients; Stage IIA, NR 1 patient, and Stage IIB, 2 R/8 patients. Aggressive NSCLC demonstrated 1) aneuploidy, Her2/neu overexpression and EGF receptor overexpression in the same cells, 2) overexpression of Her2/neu and VEGF, 3) underexpressed EGF receptor and VEGF. Repetitive cell based, multiparameter LSC analysis identified the majority of low stage NSCLC carcinomas having an aggressive course that could benefit from chemotherapy.

1446 Metastic Granulosa Cell Tumor of the Lung: Clinicopathologic Study of 7 Cases

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Background: Granulosa cell tumors (GCT) are ovarian neoplasms of low malignant potential, prone for late recurrence. Pulmonary metastasis can pose diagnostic problems due to their rarity and occurrence after long tumor-free intervals. We reviewed the lung specimens and clinical presentations of 7 patients presenting with metastatic GCT 6 to 18 years after removal of the primary ovarian tumor.
Design: From 1976-2003 seven cases of metastatic granulosa cell tumor of the lung were retrieved via the Mayo Clinic computerized medical records database and personal consultation materials. Clinical information was obtained from patient notes, clinicians treating the patients, and pathologists referring the cases. H & E sections of all lung and available ovarian specimens were reviewed. Immunoperoxidase studies were performed on selected paraffin blocks from the lung specimens, using antibodies directed against the following antigens: vimentin, desmin, smooth muscle actin, cytokeratin AE1/AE3, CAM5.2, EMA, S-100, HMB-45, CD34, chromogranin, synaptophysin, TTF-1, calretinin, inhibin, estrogen receptor, and progesterone receptor.
Results:

		Clinical Findings			
Age	Ovarian Tumor Diagnosis	Interval to Lung Metastasis (Y)	Gross Features	Knowledge of Prior Ovarian Tumor	Referring Diagnosis of Lung Lesion(s)
58	Ovarian carcinoma	18	Multiple (0.6-2.5 cm)	Yes	Poorly differentiated small cell neoplasm
45	Small cell ovarian carcinoma	8	Solitary (2.5 cm)	Yes	Unclassifiable lung neoplasm
71	Granulosa cell tumor	17	Multiple (1.5-1.8cm)	No	Unclassifiable lung neoplasm
52	Recurrent pelvic granulosa cell tumor	6	Solitary (3.0 cm)	Yes	N/A
61	Granulosa cell tumor	-2	Solitary (3.0 cm)	No	Grade 4 Adenocarcinoma
80	Recurrent ovary-pelvic granulosa cell tumor	8	Multiple (1.5-4.0 cm)	Yes	N/A
63	Granulosa theca cell tumor	25	Solitary (replacing entire lobe)	No	Unclassifiable lung neoplasm

Histologic and immunohistochemical findings Metastatic granulosa cell tumor of the lung shows monomorphic epitheliod cells with round to ovoid pale nuclei, nuclear groove, inconspicuous nucleoli, and scant cytoplasm, mainly in a diffuse or trabecular growth pattern, with positivity for vimentin, inhibin, and calretinin.
Conclusions: Pulmonary metastases from ovarian GCT are uncommon and may occur after long tumor-free intervals. This may cause diagnostic problems, especially if clinicians and/or pathologists are unaware of a prior diagnosis of GCT. In this study, the lung metastases were initially incorrectly diagnosed in all patients, emphasizing the need to review clinical history and previous surgical specimens in patients with unusual lung neoplasms.

1447 Concurrent Expression of VEGFC, VEGFR3 and VEGFR2 Is Related to Tumor Cell Proliferation and Lymph Node Metastasis in Resected Non-Small-Cell Lung Cancer

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Background: Resected non-small-cell lung cancer (NSCLC) has a high risk of relapse and lymph node (LN) metastases is still their major prognostic factor. A poor prognosis has been associated with Vascular Endothelial Growth Factor Receptor 3 (VEGFR3) and VEGFC expression in tumor cells. This study was designed to evaluate in NSCLC, the expression of Vascular Endothelial Growth Factor C (VEGFC) and its receptors VEGFR2 and VEGFR3, and to investigate their relation with histology, LN metastases status and proliferation.
Design: Ninety-six consecutive patients with resected pathological stage I-IIIA NSCLC without preoperative chemotherapy were reviewed. Half of them were classified as pN1-2. Expression of VEGFC, VEGFR2, and VEGFR3 was evaluated by immunohistochemistry on paraffin-embedded sections. Mitotic index (MI) and Ki67 expression were assessed to evaluate tumor proliferation.
Results: VEGFR2, VEGFR3, and VEGFC were respectively expressed in 56%, 73% and 41% of the tumors. VEGFR3 and VEGFC expressions were associated with squamous cell carcinoma histology (respectively p<0.0001 and p=0.03) and VEGFR3 expression was associated with LN metastases (p=0.0052). Coexpression of VEGFC and VEGFR3 and/or VEGFR2 was observed in 41 tumors (65%). Coexpression of VEGFC, VEGFR2 and VEGFR3, was observed in 24 tumors (25%). The relation between VEGFC, VEGFR2 and VEGFR3 expression, MI, Ki67 expression and LN metastasis is figured in Table 1.
Conclusions: A potential role of autocrine and/or paracrine VEGF pathways can be hypothesized in NSCLC for the VEGFC/VEGFR3/VEGFR2 loop as their concurrent expression was frequent and associated with a high proliferation rate and LN metastases.

Table 1: Relation between VEGFC, VEGFR2, VEGFR3 expression, mitotic index (MI), Ki67 expression and lymph node (LN) metastasis

	Proportion of tumors*	MI	Ki67	Lymph node metastasis
VEGFC + VEGFR2 - VEGFR3 -	14%	7.6	26%	16.5%
VEGFC + VEGFR2 - VEGFR3 -	14%	7.7	31%	41.5%
VEGFC + VEGFR2 + VEGFR3 -	23%	13.3	45%	40%
VEGFC + VEGFR2 + VEGFR3 +	27.5%	31.6	55%	66.5%
VEGFC + VEGFR2 - VEGFR3 +	12.5%	30.5	52%	72.7%

* 9% of the tumors were negative for VEGFC and expressed either VEGFR2 or VEGFR3

1448 Telomere Length and Telomerase Expression in Small Non-Mucinous Bronchioloalveolar Carcinoma (BAC) of the Lung

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Background: Telomeres are located at the ends of every human chromosome, and are subject to shortening in each cycle of cell division in cell senescence and early carcinogenesis. In contrast, cancer cells show no loss of telomere length, due to the telomere-stabilizing action of the enzyme telomerase. BAC shows growth of neoplastic cells along pre-existing alveolar structures without evidence of stromal, vascular, or pleural invasion. Using archival and frozen tissue samples (41 small BAC measuring 2 cm or less in greatest diameter, and 8 small mixed subtype adenocarcinomas measuring 2 cm or less in greatest diameter), we performed fluorescent in situ hybridization (FISH) and Southern blotting for telomere length, and ISH for human telomerase reverse transcriptase (hTERT) mRNA, and looked for relations to adenocarcinoma development.

Design: We examined (a) the expression of telomeric DNA (using an FITC-labeled telomere-specific peptide nucleic acid probe) in all 41 BACs and 8 mixed subtypes, (b) telomere length (utilizing Southern analysis) in 6 frozen samples of small BACs and mixed subtypes, and (c) the expression of hTERT mRNA (using ISH) in 40 BACs and 6 mixed subtypes.

Results: In normal lung, stromal cells in the alveolar septa and alveolar macrophages displayed normal telomere length. The mean number of telomeric signals per nucleus (by FISH) was 16.2 in normal cells, 7.4 in BAC, and 18.7 in mixed subtypes. Statistically significant differences in mean number of telomere signals could be shown between BAC and mixed subtypes, and between BAC and normal cells (each, $p<0.0001$). In normal tissue, the number of telomere signals tended to be linearly proportional to average telomere length, as assessed by Southern blotting. Positive expressions of hTERT mRNA were recognized in 98% of BAC and 100% of mixed subtypes.

Conclusions: We have demonstrated telomere shortening in small non-mucinous BAC, indicating the earliest phase of pulmonary carcinogenesis. The expressions of telomeric DNA and telomerase would appear to be of great importance in the development of pulmonary adenocarcinomas.

1449 Eukaryotic Initiation Factor-4E (eIF-4E) and Cyclin D1 Expressions Associated with Patient Survival in Lung Cancer: A Study Utilizing High-Density Tissue Microarray and Immunohistochemistry

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Background: eIF4E, a key member of eukaryotic initiation factor family, controls various processes associated with polyamine synthesis, cell cycle progression, activation of proto-oncogenes, and angiogenesis. Cyclin D1 also plays a role in cell cycle progression. Although expression of Cyclin D1 by human tumors has been extensively studied, expression of eIF4E in non-small cell lung cancer (NSCLCs) has received little investigation.

Design: Four tissue microarrays (TMAs) were constructed from 190 NSCLCs. Immunohistochemistry was performed using an avidin-biotin complex method and monoclonal antibodies against eIF4E (clone 87, Dako) and cyclin D1 (clone DCS-6, Dako). For each stain, a score of 0-3 was assigned to each tumor based on the mean percentage of stained neoplastic cells and the mean staining intensity of tumor cells (0=no staining, 1=weak, 2=moderate, 3=strong). Key clinical information including patient survival, as well as tumor stage, smoking history, performance status, weight loss, histology grade, and lymph node involvement, was obtained. Correlation of expression of markers and clinicopathologic variables was statistically analyzed using Spearman rank correlation and Kaplan-Meier analysis.

Results: For eIF-4E, 91.2% of tumors demonstrated positive expression. For cyclin D1, 70 cases (64%) were positive. Positive eIF-4E correlated with significantly shorter patient survival ($p=0.03$). In contrast, positive cyclin D1 correlated with longer patient survival ($p=0.014$). Moreover, among cyclin D1 positive group, patients who had positive eIF-4E, had shorter survival ($p=0.012$).

Conclusions: These results indicate that both cyclin D1 and eIF-4E are commonly expressed NSCLC. The adverse outcome in patients with overexpression of eIF-4E suggests its potential prognostic role in NSCLC. This study warrants a further investigation to explore the value eIF-4E in identifying patients with aggressive disease for adjuvant treatments.

1450 Clinicopathologic Analysis of 61 Cases of Thymic Neoplasms: Is the WHO Classification Scheme Practical?

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Background: The World Health Organization (WHO) classification scheme of thymic neoplasms has been a controversial issue. In this study, we present the clinical and histologic features of 61 thymic neoplasms and evaluate the significance of this classification in view of the prognosis.

Design: A total of 61 patients with thymoma who underwent resection in Buffalo area hospitals, Buffalo, NY, between 1992 and 2005 were reviewed retrospectively. The histologic subtype of the cases was determined according to the new WHO classification. The stage was also determined according to modified Masaoka's staging system as stage I, II, III, and IV. Interface eosinophilic infiltrate of tumor was recorded as either positive or negative.

Results: Fifty seven patients had available long follow up records. Histologically, there were 6 type A, 15 type AB, 7 type B1, 6 type B2, 17 type B3, and 9 type C tumors. Masaoka's stages were 30 stage I, 14 stage II, 8 stage III, and 5 stage IV. Eosinophilic infiltrate was seen in 7 of 9 subtype C cases, and 1 of 52 cases of the other types ($p<0.001$). A close correlation between histologic subtype and stage ($p<0.001$) was seen. While B3 subtype showed worse outcome than A, AB, B1 and B2 subtypes ($p<0.001$), Masaoska's stage had no significant correlation with the clinical outcome.

Conclusions: The WHO histologic classification scheme significantly correlated with clinical outcome. It is of great clinical value to appropriately diagnose B3 subtype. Eosinophilic infiltrate could be useful histologic marker to differentiate between thymoma and thymic carcinoma (type C).

1451 The Assessment of Cytology and Fluorescence In Situ Hybridization for the Detection of Early and Advanced Stage Lung Cancers in Bronchoscopically Obtained Brushing Specimens

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Background: In 2005, approximately 172,500 people in the United States will be diagnosed with lung cancer and 160,400 will die of this disease. The dismal 5-year survival of 15% for all patients diagnosed with lung cancer has been attributed to the fact that most patients have advanced stage of disease at the time of diagnosis. The goal of this study was to prospectively determine the relative sensitivity and specificity of conventional cytology and fluorescence in situ hybridization (FISH) for the detection of early and advanced stage lung cancer in brushing specimens obtained from patients undergoing bronchoscopy.

Design: This study analyzed the utility of FISH using the LAVysionÒ probe set (contains probes to centromere 6, 5p15, 8q24 (*C-MYC*) and 7p12 (*EGFR*)) to detect lung cancer in comparison to conventional cytology. FISH cases were considered positive if there were five or more cells with gains of two or more chromosomes or amplification of either the *C-MYC* or *EGFR* regions. Seventy-eight patients with concurrent FISH and cytology results whose specimens were collected during bronchoscopy were evaluated.

Results: Using bronchoscopic biopsy, surgical resection, and/or transthoracic needle aspirate results as the gold standard, the relative sensitivity of FISH and cytology on brushings was 74.5% (38/54) and 50.0% (27/54) ($P=0.007$), respectively. The relative sensitivity's of FISH and cytology for early non-small cell (stage I and II) and advanced non-small cell (stage III and IV) lung cancers were 71.9% (23/32) vs. 53.1% (17/32) ($P=0.008$); and 73.3% (11/15) vs. 53.3% (8/15) ($P=0.250$), respectively. There was no significant difference observed in the specificity of cytology and FISH among patients without evidence of cancer (N= 24; 95.8% vs. 79.2%; $P=0.125$).

Conclusions: The results of this study suggest that FISH is significantly more sensitive than and nearly as specific as conventional cytology for detecting lung cancer, especially early stage lung cancer, on bronchial brushings specimens collected during bronchoscopy.

1452 Vitamin D Receptor and Steroid Receptor Coactivator-1 Expression in Pulmonary Neuroendocrine Neoplasms

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Background: Vitamin D derivatives have been shown to bind to a receptor (VDR) on neoplastic cells and induce differentiation. Steroid receptor coactivator-1 (SRC-1) interacts with VDR in a ligand-dependent manner for nuclear receptor-mediated transcription. The expression of VDR and SRC-1 in pulmonary neuroendocrine neoplasms has received little investigation.

Design: Immunohistochemical staining for VDR (1:200; Biomeda) and SRC-1 (1:50; Novus Biologicals) was performed on tissue microarray slides composed of triplicate punch samples of 52 carcinoids, 11 large cell neuroendocrine carcinomas (LCNECs), and 42 small cell carcinomas (SCCs). Nuclear staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate) or 3+ (strong); the percentage of tumor cells staining was scored as 0 (< 10%), 1 (10-50%) or 2 (> 50%); and a combined score was calculated from the average intensity and percentage of cells staining for each tumor. Information about the presence or absence of tumor metastases was available for 29 patients. Statistical analysis was performed using non-parametric tests of significance.

Results: VDR expression was noted in 96.2% of the carcinoids (mean score: 2.4), 81.8% of the LCNECs (mean score 1.4), and 50.0% of the SCCs (mean score: 0.8). Expression rates and mean scores for SRC-1 expression in the LCNECs (81.8% and a mean score of 1.6) and SCCs (56.4% and a mean score of 1.1) were similar to those of VDR, but SRC-1 expression in carcinoids was substantially lower (34.0% and a mean score of 0.6) than VDR. Reduced expression of VDR correlated significantly with metastasis ($p=0.03$), but no significant correlation between SRC-1 and metastasis was observed. In addition, there was no significant correlation between VDR and SRC-1 expression.

Conclusions: Expression of VDR is inversely related to pulmonary neuroendocrine tumor grade and likelihood of metastasis. SRC-1, however, shows no apparent association with metastasis. Despite the close biologic interaction between VDR and SRC-1, this study shows no significant correlation between VDR and SRC-1 expression, which may be due to involvement of other coactivators or mediators in this process.

1453 Evaluation of Expression Characteristics of p-Akt, Pax-5, Ship-1, ALK-1 and TGF-β Protein Molecules in Pulmonary Neuroendocrine Tumors (NT)

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Background: Akt is a serine/threonine kinase that leads to stimulation of cell cycle progression, cell proliferation, and inhibition of apoptosis. Pax-5 is a B cell specific transcription factor crucial for B cell ontogeny. Ship-1 is involved in the regulation of M-CSF receptor-induced Akt activation. ALK-1 is a component of the TGF-β receptor complex, and TGF-β is able to modulate angiogenesis and vascular remodeling. Expression of p-Akt, Pax-5, Ship-1, ALK-1 and TGF-β, and their roles in pulmonary NT have not been reported. We examined the expression of p-Akt, Pax-5, Ship-1, ALK-1 and TGF-β in pulmonary NT, and evaluated the correlations among expression characteristics, each histologic subtype and clinical significance of these molecular markers.

Design: We evaluated the p-Akt, Pax-5, Ship-1, ALK-1 and TGF-b expressions in 45 cases of typical carcinoid (TC), 4 cases of atypical carcinoid (AC), 10 cases of large cell neuroendocrine carcinoma (LCNEC), and 41 cases of small cell carcinoma (SCC) using tissue microarray.

Results: p-Akt was positively expressed in 1/45 (2%) of TC, 0/4 (0%) of AC, 9/10 (90%) of LCNEC and 28/41 (68%) of SCC. Pax-5 was positively expressed in 6/45 (13%) of TC, 3/4 (75%) of AC, 7/10 (70%) of LCNEC and 14/41 (34%) of SCC, respectively. Ship-1 molecule was positively expressed in 2% of TC, 0% of AC and LCNEC, and 10% of SCC, respectively. ALK-1 and TGF-β molecules were not expressed in any cases of pulmonary NT.

Conclusions: The p-Akt expression was markedly increased in cases of LCNEC and SCC, and Pax-5 was frequently expressed in cases of AC and LCNEC. However Ship-1, ALK-1 and TGF-β were not well expressed in pulmonary NT. This study suggests that p-Akt can be a good predictor of highly malignant NT, and therefore, p-Akt may be a potential novel therapeutic target for the management of SCC and LCNEC.

1454 Coexpression of c-kit and bcl-2 in Small Cell Carcinoma and Large Cell Neuroendocrine Carcinoma of the Lung

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Background: It has been shown that tyrosine kinase oncoprotein c-kit and anti-apoptotic molecule bcl-2 are overexpressed in several types of malignant tumors, including small cell carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC) of the lung. Coexpression of these two molecules in the lung neuroendocrine tumors (LNETs) has not been documented. Here we analyzed immunostaining results to determine coexpression patterns of c-kit and bcl-2 in the spectrum of LNETs.

Design: Thirty-six cases of surgically resected LNETS, including 10 typical carcinoids (TC), 5 atypical carcinoids (AC), 7 SCLCs, and 14 LCNECs, were immunohistochemically studied using polyclonal antibody against c-kit and monoclonal antibody against bcl-2. A tumor was recorded positive if more than 10% of the tumor cells showed immunoreactivity. A p value of <0.05, as determined by Fisher exact test, was considered statistically significant.

Results: The results are summarized in the table. There was a progressive increase in the frequency of c-kit and bcl-2 expression and coexpression from carcinoid tumors through LCNEC to SCLC. High grade neuroendocrine carcinomas (HGNECs) were more likely to coexpress c-kit and bcl-2 when compared with low grade carcinoid tumors (p<0.01). Among the HGNECs, SCLCs were more likely to coexpress these two proteins than LCNECs (p<0.05).

Conclusions: 1. Both c-kit and bcl-2 are significantly overexpressed in HGNECs in comparison to carcinoid tumors. 2. Coexpression of c-kit and bcl-2 is more common in SCLC. 3. High frequency of coexpression of these two molecules in HGNECs suggests that they might be linked in the carcinogenic pathway, given their important roles in carcinogenesis.

Expression and coexpression patterns of c-kit and bcl-2 in lung neuroendocrine tumors.

	c-kit +	bcl-2 +	c-kit +/bcl-2 +
TC (n=10)	0	0	0
AC (n=5)	0	1 (20%)	0
TC+AC (n=15)	0	1 (6.7%)	0
LCNEC (n = 14)	7 (50%)	9 (64%)	7 (50%)
SCLC (n = 7)	7 (100%)*	7 (100%)	7 (100%)*
LCNEC+SCLC (n=21)	14 (67%)**	16 (76%)**	14 (67%)**

*p <0.05 in comparison with LCNEC; **p<0.01 in comparison with TC+AC; +, positive staining.

1455 EphA2 Expression in Non-Small Cell Lung Cancers

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Background: The erythropoietin-producing hepatocellular (EPH) receptors represent the largest known family of receptor tyrosine kinases, which are activated by interaction with cell-surface ligands, termed ephrins. In normal cells, EphA2 expression appears to be restricted to intercellular junctions, where it binds to its ligands that are anchored to the membranes of adjacent cells. After binding, EphA2 becomes autophosphorylated resulting in a cascade of downstream signals that ultimately inhibit cell growth and migration. It has been reported that EphA2 is expressed at relatively low levels on a variety of adult epithelial cells and is associated with higher grade and lymph node metastatis in a variety of tumors. We have found a greater expression of EphA2 in well differentiated esophageal squamous cell carcinomas in the past. There are no published data comparing expression of EphA2 in different histologic subtypes of non small cell lung cancer.

Design: Tissue microarrays were created from archival paraffin embedded tissue samples from 146 patients with primary non-small cell lung cancer (NSCC), of which 68 were adenocarcinoma (including 7 bronchiolo-alveolar), 44 were squamous cell carcinoma (including 4 basaloid), 19 poorly differentiated NSCC, and 8 large cell carcinomas. In addition to the tumor, areas of normal lung parenchyma were obtained from the cases. The microarrays were stained immunohistochemically with a mouse monoclonal antibody to EphA2, clone D7.

Results: EphA2 immunoreactivity was detectable in 49 of 146 (33.6%) NSCC. Of 44 squamous cell carcinomas, 24 (54.5%) were positive; with the majority of positive staining in the well differentiated areas. Among 75 adenocarcinomas, 17 (22.7%) were positive. Six of 19 (31.6%) poorly differentiated NSCC and 2 of 8 (25%) large cell carcinomas were positive.

Conclusions: There is a greater expression of EphA2 in squamous cell carcinoma of the lung compared to adenocarcinomas (p=0.0006). Positive staining in pulmonary squamous cell carcinoma was predominantly in the well differentiated areas, as we previously reported in the esophagus. This finding is in contrast to previously published reports showing upregulation of EphA2 in a variety of poorly differentiated non-pulmonary cancers. Our findings indicate that loss of EphA2 expression may, in fact, reflect a higher grade tumor, particulary in squamous cell carcinoma. Additional studies are ongoing to determine the prognostic significance of EphA2 expression in NSCC.

1456 *EGFR* Mutation Analysis in 334 Consecutive Non-Small Cell Lung Cancers: Evidence for Frequent Amplification of the Mutant *EGFR* Allele and Survival Correlations of Mutation Type

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Background: Mutations in the epidermal growth factor receptor (*EGFR*) gene in non-small cell lung cancer (NSCLC) are associated with sensitivity to EGFR tyrosine kinase inhibitors (TKI). The two most common mutations (accounting for close to 90% of all mutations) are short in-frame deletions within exon 19 and an exon 21 point mutation resulting in the codon change L858R.

Design: Tumor DNAs from 334 patients with NSCLC, mostly extracted from paraffin blocks or unstained sections, were studied for exon 19 deletions (detected by length analysis of fluorescent PCR products) and exon 21 L858R mutations (detected by a fluorescent PCR-restriction enzyme digestion assay). These recently reported assays (J Mol Diagnostics 7:396-403, 2005) detect mutations in the presence of up to 90% non-neoplastic cells, an improvement over direct sequencing.

Results: *EGFR* mutations were found in 78/334 (23%) cases. Among the 78 cases with mutations, 55 (71%) had exon 19 deletion and 23 (29%) had the exon 21 L858R mutation; 50 (64%) were women; 31 (40%) were adenocarcinomas (ADCA) with partial or complete bronchioloalveolar carcinoma (BAC) features, 42 (54%) were ADCA without BAC features, and 5 (6%) were other types of ADCA. One unusual case showed two synchronous primary ADCA of different histology with different exon 19 alterations. Our exon 19 assay also allows comparison of the proportion of mutant and normal *EGFR* alleles and this showed that 15/55 (27%) tumors with exon 19 deletions had multiple copies of the mutant allele. The survival by *EGFR* mutation was analyzed in a subset of 34 patients with metastatic NSCLC treated with EGFR TKIs, of which 32/33 (97%) had an initial response. The overall survival and time to progression of the 20 patients with exon 19 deletion were longer than those of the 14 with the exon 21 mutation (both p=0.01).

Conclusions: Our high percentage of *EGFR* mutations may reflect referral bias for ADCA histology. The ratio of exon 19 to 21 mutations was higher than in previous studies based on direct sequencing (>2:1 vs 1:1), suggesting that the prevalence of exon 19 deletions may be underestimated by direct sequencing. Amplification of the mutant *EGFR* allele is common, at least for exon 19 deletions. Tumors with exon 19 and 21 mutations may have slightly different clinical courses in response to EGFR TKIs.

1457 Snail and Slug Expression in Lung Carcinomas

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Background: The zinc finger transcriptional factors Snail and Slug have been implicated in tumor progression and metastasis. These factors are believed to repress E-cadherin expression and mediate epithelial-mesenchymal transition and invasiveness. A small number of studies have addressed their expression in carcinomas of the breast, ovary, liver and esophagus, but little information is available regarding these factors in lung cancers.

Design: Tissue microarrays containing triplicate punch samples of 350 non-small cell lung cancers (NSCLCs) and 36 small cell carcinomas (SCCs) were immunostained for Snail (1:10; Santa Cruz) and Slug (1:100; Santa Cruz). Staining intensities were graded as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong), and the percentages of tumor cells staining were assessed. For each tumor, mean values for staining intensity and percentage were calculated, and used to derive an overall score of 0-3. Kaplan-Meier analysis and non-parametric tests of significance were used to evaluate correlations with survival, stage, smoking history, and histologic type and grade, for the NSCLCs, and with metastasis for the SCCs.

Results: Cytoplasmic staining for Snail and Slug was observed in 92.9% and 95.5% of NSCLCs, respectively, and 67.6% and 38.9% of SCCs, respectively. Purely nuclear staining was only noted in SCCs: Snail-20.6% and Slug-30.6%. Combined nuclear and cytoplasmic staining occurred in 8.8% (Snail) and 16.7% (Slug) of SCCs and 1.7% (Slug) of NSCLCs. For NSCLCs, Snail expression was significantly associated with higher histologic grade (p=0.009) and pack-years of smoking (p=0.05), and there was a trend towards association with higher stage (p=0.06). Slug expression declined with increasing tumor grade (p< 0.0001) and stage (p=0.04). Although histologic cell type of NSCLC showed no relationship to expression of Snail, Slug expression was significantly higher in adenocarcinomas than squamous cell carcinomas (p<0.0001). Expression of Snail and Slug did not correlate significantly with five-year survival for NSCLCs, or metastasis for SCCs.

Conclusions: Snail and Slug are widely expressed in NSCLCs and SCCs, and variations in their expression patterns show relationships to tumor stage, histologic type and grade, and smoking history, but not five-year survival.

1458 Distribution, Density and Morphology of Langerhans' Cells in the Lung: Relationship to Lung Development, Pulmonary Fibrosis and Smoking

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Background: Most interest in Langerhans cells (LCs) of the lung has centered on LC histiocytosis (LCH), but the distribution, cellular characteristics and function of the cells remains relatively unexplored. We systemically examined their distribution, density and morphology and their relationship to lung development, pulmonary fibrosis and smoking.

Design: Lung tissue specimens were collected from autopsy and surgical materials. There were 30 cases in infants, 6 in older children, 17 in adults with diffuse alveolar damage (DAD) (all non-smokers), 8 in cases with advanced pulmonary fibrosis (APF), and 32 in lung cancer patients (smokers 17 cases, non-smokers 15 cases). We performed immunohistochemical studies using polyclonal S-100 protein antibody (Dako) and CD1a antibody (Novocastra Lab) on formalin-fixed and paraffin-embedded lung tissues. LCs were distinguished from other S-100 protein-positive cells by their dendritic nature. Semiquantitative attempt was done by using average numbers per high-power field as follows: 1+ (fewer than 2 cells); 2+ (3-5 cells); 3+ (6 or more cells).

Results: LCs varied from region to region within the same slide. LCs were observed in multiple sites: 1) lamina propria of bronchial mucosa; 2) around and within walls of bronchi and bronchioles; 3) in interstitium around blood vessels; and 4) rarely in alveolar walls. They lay individually or in small groups and never in aggregates. Both nuclear and cytoplasmic staining was seen in most instances. The numbers in infants were 1+ in 10%, 2+ in 13% and 3+ in 77%. The numbers in children were 1+ in 17%, 2+ in 66% and 3+ in 17%. The numbers in DAD were 1+ in 59%, 2+ in 35% and 3+ in 6%. The numbers in APF were 1+ in 0%, 2+ in 38% and 3+ in 62%. The numbers in lung cancer in smokers (versus non-smokers) were 1+ in 18% (versus 73%), 2+ in 47% (versus 27%) and 3+ in 35% (versus (0%).

Conclusions: The density of LCs was significantly higher in infants, in APF and in smokers with lung cancer compared to controls. The increase in infants compared to adults suggests that LCs have a role in lung development. There is a strong relationship of hyperplasia of LC to pulmonary fibrosis and to smoking of cigarettes in patients with lung cancer but not in non-smokers with lung cancer. The discordance between lung cancer patients who do or do not smoke cigarettes is unexplained.

1459 Role of Lymphangiogenesis in Repair and Remodeling of the Lung after Acute Lung Injury

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Background: Diffuse alveolar damage (DAD) and advanced pulmonary fibrosis (APF) represent lung injury with repair and remodeling. The lymphatic vasculature is critical for regulating tissue fluid, but its participation in DAD and APF has not been studied in detail. Recently, vascular endothelial growth factor-D (VEGF-D) is known as a factor in stimulating lymphangiogenesis. To examine the roles of lymph vessels in lung remodeling, we compared the distribution and intensity of VEGF-D and VEGF receptor-3 (Flt-4) in certain disease states to normal.

Design: Immunohistochemical expression patterns of podoplanin, D2-40, CD34, Flt-4 and VEGF-D were examined in formalin-fixed and paraffin embedded sections of lung from 25 autopsies with DAD (10 exudative stage, 11 organizing stage, 4 fibrotic stage) and from 10 autopsies with APF and in normal lugs as controls. After microwave antigen retrieval all cases were immunostained with an anti-podoplanin (AngioBio), anti-D2-40 (Dako), QBEnd 10 (Dako), anti-Flt-4 and anti-VEGF-D (R&D Systems) antibodies using a standard indirect avidin-biotin horseradish peroxidase method.

Results: In controls lymph vessels were very few and seen in the peribronchial, periarterial and interlobular septal regions. Immunoexpression of podoplanin and D2-40 in the study patients showed a similar distribution, reflecting the normal anatomic distribution of lymphatics in the lung. Vessels positive for these antibodies decreased in number in exudative stage of DAD and increased in number with progression of fibrosis from organizing to fibrotic stage. New lymphatics were most prominent in APF. Flt-4-positive vessels were identified in the endothelium of not only D2-40 and podoplanin positive lymph vessels but also CD-34-positive blood vessels. The cytoplasmic expression of VEGF-D was observed in bronchial and bronchiolar epithelium and regenerated type 2 pneumocytes in every stage of DAD and in APF. In controls immunoexpression of VEGF-D and Flt-4 was seldom seen.

Conclusions: The number of lymph vessels and the intensity of their staining with the markers studied increased with progression of fibrosis after DAD and were most prominent in APF. Regenerating epithelial cells and pneumocytes also showed marked immunoexpression of VEGF-D. These findings suggest that lymphatics and lymphangiogenesis may have a role in the repair and remodeling of the lung after acute lung injury.

1460 The Lung Pathology of Cocaine Paste (Basuco) Smoking in Bogota, Colombia

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Background: Refined cocaine hydrochloride is used by inhalation or intravenous injection. It is not suitable for smoking, as in this form it is decomposed by heat. In Colombia, the first crude product extraction after the treatment of coca leaves is known as coca paste or basuco, and therefore, it still has solvents and contaminants such as gasoline and manganese. It is an alkaloid and sulfatated form, which is resistant to heat, and thus, it can be smoked in a cigarette form allowing for a much more rapid and efficient absorption than nasal inhalation. One characteristic feature that identifies heavy abusers of basuco is the presence of severe chronic burns in their fingers as they usually consume the entire basuco cigarette holding the very last part of it with the tips of the fingers. Coca paste is different from crack, which is cocaine that is altered for smoking after it has been purified. Crack is of more popular use in the United States. The lung histologic findings after abuse of basuco are not well described.

Design: Ten unidentified young adult men who died because of violent episodes and had extreme finger stigmata of habitual basuco (cocaine paste) smoking were autopsied. In these individuals, the fingers were severely burned and had the characteristic charred appearance known to the local authorities as indicative of heavy smoking basuco abusers. The lungs in these individuals were examined by light and electron microscopy.

Results: The most remarkable finding was the presence of widespread deposits of histiocytes in alveolar and bronchiolar spaces. The histiocytes contained brown pigment with an abundant presence of black particles that varied in shape from pinpoint deposits to irregular polygonal forms. Although the amount of inflammation around the airways was minimal, the alveolar interstitum showed lymphocytes. Ultrastructurally, the alveolar septae were denuded of type I pneumocytes. There was proliferation of type II pneumocytes with abundant cytoplasmic vacuoles without lamellar bodies. There were numerous alveolar macrophages containing phagolysosome vacuoles with electro-dense zones suggestive of metal deposits and electro-lucent areas with spicule shapes corresponding to ghosts of cellular membranes.

Conclusions: The smoking of coca-paste (basuco) produces a desquamative pneumonia-like injury with interstitial changes and mild airway inflammation similar to cigarette smoking. However, the histiocytes in alveolar spaces are far more numerous and contain more conspicuous metal impurities.

1461 A Lung Adenocarcinoma Molecular Subtype Associated with Non-Smokers and EGFR Mutations

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Background: The most common recognized cause of lung cancer is cigarette smoking, however, about 15% of patients have never smoked. It is likely that lung cancer molecular subtypes are strongly influenced by the predisposing carcinogenic exposures. The assumption is, and limited data suggest, that the molecular mechanisms contributing to the biology of tumors in non smokers differs from those of smokers. Defining the molecular features of cancers from non-smokers will contribute to improved understanding of lung cancer carcinogenesis and classification.

Design: 106 primary lung adenocarcinoma were analyzed by transcript profile (Affymetrix U133A), mutational profile (EGFR, KRAS, ERBB2 and BRAF) and immunoreactivity for EGFR, Her2/neu, ErbB3, ErbB4, c-kit, p-Akt, p-Erk, ER, PR, Ki67 and caspase-3. Tumors were reviewed for histopathological classification, grade, histological components and local invasion. Correlation was made with clinical features including age, smoking history, gender, and survival. Statistical analysis was performed using SPSS v 13.

Results: Unsupervised clustering analysis of transcript profiles revealed a robust lung adenocarcinoma molecular subgroup of 16 cases (reproducibility index = 0.914) strongly associated with never smoking or less than a 10 pack year history of smoking ($p<0.001$). This molecular subgroup correlated strongly with EGFR mutation ($p<0.001$) and also over expression of TTF1 ($p=0.027$) and p-AKT ($p=0.023$); presence of a papillary component ($p=0.001$) and presence of micropapillary features ($p=0.011$); invasion in scar ($p=0.049$) and size of invasion ($p=0.006$); and age ($p=0.034$). No mutations of KRAS, ERBB2, or BRAF were identified in this subgroup. No correlation was found with gender, survival, grade, presence of an acinar, bronchioloalveolar or solid component, scar size, vascular invasion, or expression of EGFR, HER2/neu, ErbB3, ErbB4, Ki67, p-ERK, c-kit, ER, PR, and caspase-3.

Conclusions: Our data suggest there is a lung adenocarcinoma molecular subtype that commonly arises in non-smokers and has distinctive clinicopathologic and molecular features. This subgroup has transcript profiles similar to patients with activating mutations of EGFR implicating the EGFR signaling pathway regardless of EGFR status. Our initial studies also suggest that these subtypes are associated with distinct pathologic features. Although independent validation is needed, these studies help define a subclass of lung adenocarcinoma for which EGFR mediated signal transduction may be a relevant therapeutic target.

1462 Occult Aspiration of Gastric Contents in Biopsy/Resection Specimens: An Often Unrecognized Cause of Lung Infiltrates and Nodules

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Background: Aspiration pneumonia is a well recognized finding in debilitated patients at autopsy but is uncommon in biopsy specimens. We have encountered a surprising number of cases in surgical specimens, most of which were unsuspected clinically and pathologically. This study was undertaken to clarify clinical and pathologic features of aspiration pneumonia in surgical specimens and to identify predisposing factors.

Design: 57 surgical specimens of aspiration pneumonia were identified in consultation files between 1995 and 2005. Hematoxylin and eosin-stained slides were evaluated and clinical data reviewed.

Results: Patients ranged from 2 to 85 years old (mean 55), with a male:female ratio of 2:1. The most common presenting symptoms were dyspnea (14), fever (10) and cough (5). A history of recurrent pneumonia was present in 7. Radiographically, bilateral infiltrates or nodules were found in 19 cases and solitary masses suspicious for neoplasm in 11. Aspiration was suspected clinically in only 4 patients. Predisposing factors for aspiration were identified in 30 (50%) cases, including esophageal surgery, dilatation,

dysmotility, hernias or carcinoma (9), gastric bypass, stapling, obstruction or carcinoma (8), drug abuse (6) and various neurological conditions (6). The most common referral histopathologic diagnoses were BOOP (7), usual interstitial pneumonia (4), BOOP-like reaction (3), organizing pneumonia (3), diffuse alveolar damage (3) and bronchocentric granulomatosis (3). Histologically, bronchiolitis obliterans-organizing pneumonia (BOOP) was present in most (50) cases, acute bronchopneumonia in 3 and peribronchiolar scarring in 2. Foreign-body granulomas with multinucleated giant cells were common (44) and intrabronchiolar suppuration was found in 32. All cases showed foreign material within bronchioles and/or alveoli surrounded by multinucleated giant cells and/or neutrophils. Vegetable material or food remnants were identified in 47 cases, talc in 6, Crospovidone in 4, Kayexalate in 2 and other foreign material in 8.
Conclusions: Aspiration pneumonia is a more common cause of lung infiltrates and nodules than generally appreciated. The presence of multinucleated giant cells and/or granulomas in the background of a BOOP-like process should suggest aspiration pneumonia, and when combined with foreign material is diagnostic. A history of upper gastrointestinal tract surgery or abnormalities, neurological conditions or drug abuse is an additional clue to the diagnosis.

1463 Pulmonary Interstitial Fibrosis in Smokers Results from Inorganic Dust Exposure Rather Than Smoking: An Analysis Using Light Microscopy (LM), Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS)

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Background: It has been claimed that smoking is the cause of fibrosis in smokers. However, the role of small inorganic dusts particulates, which may play a role in pulmonary fibrosis, has not been adequately examined. Respiratory bronchiolitis (RB) is an accumulation of pigmented macrophages within respiratory bronchioles in smokers. The hypothesis is that mineral dust exposure is a risk factor for development of fibrosis independent of smoking. We tested this hypothesis by studying the relationships between smoking, RB, inorganic dusts and histologically identifiable interstitial fibrosis.
Design: 34 subjects with open lung biopsies were prospectively enrolled in the study. Clinical evaluation included a detailed general health and occupational questionnaire, pulmonary function tests and radiological studies. The following parameters were graded by LM: RB, fibrosis and particulate dust (opaque and birefringent). Semi-quantitative analysis of dust particles in the lungs was done using SEM/EDS.
Results: 16 subjects were excluded because of inadequate lung parenchyma for analysis. Among the other 18 subjects (13 females, 5 males; median age: 58 years; range: 26-78 years), there were 4 active smokers [mean pack-years (py): 83.7], 10 ex-smokers [mean py: 65.5] and 4 never smokers. RB was present in 15 subjects and absent in 3. Statistically significant associations (bivariate logistic regression analysis) were found between smoking and RB (p= .03) but not between smoking and fibrosis nor between RB and fibrosis. Fibrosis was statistically significantly associated with aluminum silicate (p= .02), silica (p= .01) and titanium (p= .005) concentrations.
Conclusions: Our results support the hypothesis that inorganic dust exposure is an independent risk factor for development of fibrosis in smokers. The size of our study group does not allow conclusions as to the interaction of smoking and dust particle exposure in the development of fibrosis.

1464 Non-Small Cell Lung Carcinomas: Expression of Total Hsp27 and p-Hsp27; a Possible Target for siRNA Therapy

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Background: Heat shock proteins (Hsps) are overexpressed in many cancers and, in their role as molecular chaperones, are cytoprotective. Hsp27 is a ubiquitously expressed small Hsp that is upregulated at the transcription level in response to various stresses including heat shock, anticancer drugs, radiation, and oxidative stress. Phosphorylated (p-Hsp27) and non-phosphorylated forms exist; the former down-regulates death-receptor-mediated extrinsic apoptosis, and the latter negatively interferes with intrinsic mitochondria-mediated apoptosis. Our aim was to characterize Hsp27 and p-Hsp27 expression in non-small cell lung carcinomas (NSCLC).
Design: Hsp 27 and p-Hsp27 were investigated in cell lines and in tissue microarrays (TMA) of NSCLC. TMAs including the central portion and invasive front of NSCLC, and adjacent alveoli and airway mucosa, were stained with mouse monoclonal anti-Hsp27 and rabbit polyclonal anti-p-Hsp27. Antibody expression was scored from 0 (no staining) to 3+ (strongest). Expression was compared in multiple areas (tumor center v. invasive front v. alveoli v. airway) within a specific tumor type, and between the center of different tumor types.
Results: Hsp27 and p-Hsp27 were expressed in all (10/10) NSCLC cell lines. Cell lines treated with siRNA against Hsp27 showed decreased growth and viability. Hsp27 and p-Hsp27 were expressed (score ≥ 1+) significantly more frequently in array tumors (center and invasive front) than in normal alveoli, and both were overexpressed (score≥ 2+) significantly more frequently in tumors (center and invasive front) compared to normal alveoli and airway mucosa. A larger number of tumors overexpressed total Hsp27 than p-Hsp27. Expression/overexpression of Hsp27 and p-Hsp27 did not differ significantly between the centers and invasive fronts within each NSCLC type, or between the center of each NSCLC type. Airway mucosa did express/overexpress Hsp27 and p-Hsp27 significantly more frequently than alveoli.
Conclusions: Overexpression of Hsp27 and p-Hsp27 was observed in NSCLC cell lines, and in the centers and invasive fronts of all types of NSCLC in TMA, and does not differ significantly between each type. Overexpression is likely related to the ability of tumors to survive via both the intrinsic and extrinsic apoptotic pathways. Our data support further investigation of these Hsps as potential therapeutic targets in NSCLC.

1465 Expression of Claudins 3, 4 and 5 in Non Small Cell Lung Cancers

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Background: Claudins are a family of adhesion molecules that serve to regulate the barrier function of tight junctions in epithelial and endothelial cells. Loss of normal tight junction function constitutes a hallmark of human carcinomas. Although claudins are expressed variably by different tissues, their role in tumorgenesis has not been extensively examined. We investigated the prognostic significance of claudins 3, 4, and 5 expression in non-small cell lung cancers (NSCLC).
Design: Formalin-fixed, paraffin-embedded sections from 113 NSCLC, including 37 squamous cell carcinomas (SCC), 43 adenocarcinomas (AC), and 33 bronchoalveolar carcinomas (BAC), were immunostained by automated methods (Xmatrx, BioGenex Laboratories, Inc, San Ramon, CA) with antibodies to claudins 3, 4, and 5 (Zymed, San Francisco, CA). The staining pattern was semiquantitatively assessed. Claudin loss of expression was assessed using limits defined as greater than 25% or 50%. Histologic and prognostic characteristics were correlated with staining percentage.
Results: Staining for claudins 3 and 4 was membranous and present uniformly in normal bronchial epithelium. Claudin 5 was demonstrated only in normal endothelium. In NSCLC, staining was also present as a continuous membranous positivity. When defined as greater than 25% staining loss, a statistically significant loss of claudin 3 expression was identified between tumor types, showing 87% (SCC), 44% (AC), and 61% (BAC) loss respectively ($p<0.0001$). Claudin 3 loss of expression also correlated with tumor grade, showing a statistically significant greater loss of expression (when defined as greater than 50% loss) in high grade tumors (49%) than in low grade tumors (25%) ($p=0.039$). When defined as greater than 25% staining loss, claudin 4 loss of expression was significantly greater in advanced stage AC (55%) versus low stage AC (27%) ($p=0.042$). Claudin 5 loss of expression showed no association with histologic and prognostic characteristics.
Conclusions: Loss of claudin 3 expression appears to differentiate between SCC, AC, and BAC and is also associated with a higher tumor grade. Loss of claudin 4 expression in AC may be associated with advanced stage of disease. These findings suggest that tumor progression may be related to claudin expression in NSCLC.

1466 Histopathologic, Immunohistochemical and Molecular Features of Screening Spiral Computed Tomography (SCT)-Detected Lung Cancers

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Background: Although there is no evidence of the occurrence of clinically indolent lung cancers, the risk of overdiagnosis due to the high sensitivity of SCT cannot be completely excluded. Detailed studies on the pathological features of SCT-detected lung cancers, however, are still lacking.
Design: We evaluated the histopathologic features of 28 SCT-detected lung cancers radically resected at the participating Institutions between 2000 and 2004. These tumors occurred within a cohort of 1,035 volunteers aged 50 or older with a minimum of 20 pack-year index undergoing annual low-dose single-slice SCT. Tumor subtyping was performed according to the 2004 WHO classification of lung cancer and, for adenocarcinoma, by evaluating central scar diameter and invasion size according to Terasaki et al. (Am J Surg Pathol 2003;27:937-951).
Results: The tumors were diagnosed in 20 males and 8 females (range 51-74 yrs), with 20 adenocarcinomas (AC), 6 squamous cell carcinomas (SCC) and 2 large-cell neuroendocrine carcinomas (LCNEC) measuring from 0.4 to 4 cm. Overall, 20 (72%) tumors were stage IA (14 AC, 4 SCC & 2 LCNEC), 2 (7%) stage IIB (1 AC & 1 SCC), 4 (14%) stage IIIA (3 AC & 1 SCC) and 2 (7%) stage IIIB (all AC). Seventeen AC were of mixed type, with variable peripheral bronchioloalveolar (BAC) component ranging from 5 to 100% (median value 30%), 2 were solid and 1 acinar without any BAC component. Two tumors were G1, 9 G2 and 15 G3 (in two post-chemotherapy patients tumor grade was not assessable). Vascular invasion was seen in 2 SCC, 9 AC and both LCNEC. Central scar diameter and invasion size were smaller in mixed AC (median values 0.85 and 0.6 cm, respectively) than in other AC types (both 1.75 cm). Ten tumors showed nuclear p53 accumulation, 24 exhibited EGFR immunoreactivity in 15-100% tumor cells and 16 a Ki-67 labeling index higher than 30%. Five of 16 AC evaluated for K-ras mutations showed mutations at codon 12, all GGT (glycine) to TGT (cysteine) transversions.
Conclusions: Our results provide further evidence that SCT-detected lung cancers share the same of fully malignant tumors, despite the early detection and clinical stage, and that SCT screening modalities do not lead to overdiagnoses of pulmonary malignancies

1467 Molecular Evaluation of Synchronous and Metachronous Lung Carcinomas: Metastasis vs. Separate Primaries

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Background: In patients with multiple pulmonary carcinomas (CA), the separation of separate primaries versus metastasis can have important prognostic and therapeutic implications. Histology is limited in making this distinction, especially if the tumors are the same histology type. We evaluated the efficacy of molecular analysis of metachronous and synchronous lung tumors for separating two primaries versus intrapulmonary metastasis.

Design: We retrieved paraffin blocks of 40 pulmonary CAs from 20 patients. The CAs in the each patients were of same histologic type (14 adenocarcinomas, 18 squamous cell CA, 6 large cell CA, and 2 small cell CA). 12 patients had synchronous and 8 had metachronous CA. 4-8 microdissection targets from neoplasm and non-neoplastic controls were removed under stereoscopic guidance and analyzed by PCR for LOH at 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q and point mutation determination in k-ras-2 . These markers were chosen based on molecular abnormalities reported in the literature and preliminary data from our laboratory. The tumors were classified as de novo or metastasis based on 3 levels of concordance: (1) marker affected - tumors were considered concordant with 50% or more of the same mutated markers, (2) same gene copy affected, and (3) temporal sequence of mutation. We also reviewed the H and E slides for the following: special type, differentiation, presence of in situ carcinoma, pattern of growth, and subjective impression.

Results: Molecular analysis showed 7/12 synchronous and 6/8 metachronous CAs were de novo and 5/12 synchronous and 2/8 metachronous were intrapulmonary metastases. Comparison with histology is shown in the table below.

	Histology Similar	Histology Different
Synchronous De Novo	5	2
Synchronous Metastasis	1	4
Metachronous De Novo	3	3
Metachronous Metastasis	2	0

The molecular analysis results by histologic type are shown in the table below.

Histologic type	De Novo	Metastasis
SCC	7	2
Adenocarcinoma	4	3
Large cell	1	2
Small cell	1	0

Conclusions: Most of the metastatic CAs were synchronous. Histology correlated poorly with molecular findings, demonstrating the limitation of H and E examination. There was no significant difference between metastasis for the various histologic types. Mutational profiling can determinate if multiple lung CAs represent intrapulmonary metastasis or a new primary with a high degree of certainty, which can effect the treatment and prognosis of patients with multiple pulmonary CAs.

1468 D2-40 Expression in Minute Pulmonary Meningothelioid Nodules

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Background: Minute pulmonary meningothelioid nodules (MPMNs) are perivenular nodular aggregates of uniform cells with round to oval nuclei, finely granular chromatin, and eosinophilic cytoplasm. The cells are typically arranged in "zellballen"-like pattern suggestive of chemodectomas but have been found by electron microscopy and immunohistochemistry to more closely resemble meningothelial cells rather than chemoreceptor cells. Molecular studies to date have not supported a common histogenesis between meningiomas and MPMNs. D2-40 is a monoclonal antibody against a formalin-resistant epitope of podoplanin which is strongly expressed in fetal testis, mesothelium and lymphatic endothelium. Recently, we have found strong membrane D2-40 immunoreactivity in leptomeninges. In this study, we evaluated D2-40 immunoreactivity in meningiomas and MPMNs.

Design: Paraffin embedded tissue from 18 meningiomas of the central nervous system (CNS) and 12 cases of lung wedge or lobectomy resections that yielded 17 MPMNs were examined. As controls, 10 cases of carcinoid tumorlets were also included in the study. Immunohistochemical staining with D2-40 was performed using heat-induced epitope retrieval and Envision +HRP on a DAKO autostainer. Cytoplasmic staining was considered positive.

Results: Uniform cytoplasmic staining with D2-40 was observed in 17/17 (100%) of the MPMNs present in the examined twelve cases and 18/18 (100%) of the meningiomas. No D2-40 immunoreactivity was found in any of the 10 cases diagnosed as carcinoid tumorlets.

Conclusions: D2-40 is uniformly expressed in CNS meningiomas and minute pulmonary meningothelioid nodules but not carcinoid tumorlets. In addition to their morphologic similarity, meningiomas and minute pulmonary meningothelioid nodules share a similar D2-40 immunophenotype. This finding suggests a potential similar histogenesis of these nodules with meningiomas.

1469 Distinct EGFR Immunoreactivity in Terminal Respiratory Unit Type Adenocarcinoma of the Lung

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Background: Clinical trials have shown that 10-20% of non-small cell lung cancer patients respond to EGFR inhibitor gefitinib therapy. Most responders are nonsmokers and female who tend to have adenocarcinoma with bronchioloalveolar features. Certain EGFR mutations have been reported to be closely associated with clinical response to gefitinib. A recent study demonstrated that these EGFR mutations were seen in terminal respiratory unit (TRU) type adenocarcinoma. We sought to explore if there is any correlation between EGFR immunoreactivity and TRU type adenocarcinoma.

Design: We examined 127 lung tumors identified in 124 consecutive lung resection specimens from 2002 to 2004 at UCSD Medical Center. Each tumor was classified according to the current WHO criteria and all adenocarcinomas were subclassified as TRU or non-TRU type. TRU type is based on the histologic differentiation to type II cells, Clara cells and/or nonciliated bronchioles. Standard immunohistochemistry was performed on all cases using a Dako Envision system with a monoclonal EGFR antibody (Dako, 1: 150). The cases showing moderate or strong EGFR positivity in more 5% of tumor cells were defined as positive. EGFR immunoreactivity pattern was also recorded in positive cases.

Results: EGFR immunoreactivity is mainly membranous in squamous cell carcinomas but demonstrates basal cytoplasmic staining with or without some membranous accentuation in adenocarcinomas. EGFR immunopositivity is significantly more common in TRU type than in non-TRU type (P<0.0001, see table).

Conclusions: Prevalent EGFR immunoreactivity is detected in TRU type adenocarcinoma and its distinctive pattern suggests an association with a particular molecular pathway.

EGFR positivity in TRU-type adenocarcinoma, non-TRU-type adenocarcinoma, and others

	Total Cases	Mean Age	Sex (M/F)	EGFR Pos
TRU	63	65	20/43	61.9%
non-TRU	23	67	12/11	8.7%
Others	41	67	23/18	53.7%

1470 Long and Short Time Survivors of Malignant Epitheloid Mesothelioma: A Genetic and Immunohistochemical Study

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Background: Malignant epitheloid mesothelioma of the pleura is a rapid progressive tumor, which usually kills patients within 24 months after diagnosis. However, there are cases known, in which patients survive much longer. When carefully analyzed there are no morphologic differences, which might permit to predict biological behavior of these two types of epitheloid mesotheliomas.

Design: We have collected 21 malignant epitheloid mesotheliomas, where patient survival was greater than 36 months and compared these to 19 malignant epitheloid mesotheliomas with less than 36 months survival. Chromosomal comparative genomic hybridization (cCGH) was done in these cases. A tissue microarray was constructed and different markers for proliferation, such as MAPkinase, Akt, mTOR, RAB, as well as members of the Wnt-pathway were tested.

Results: By chromosomal CGH no significant differences were found between these two groups. In most cases within both groups we found less than two aberrations. Some cases had no aberrations at all, again in both groups.

Conclusions: Malignant epitheloid mesotheliomas with either short or long time survival could not be separated by their morphological appearance nor by chromosomal CGH. This might be due to the much lower resolution of chromosomal CGH, or to posttranslational modification. Therefore analysis of different pathways for growth signaling might provide better information about this difference of biological behavior.

1471 Distribution of Myoepithelial Cells in Bronchioloalveolar Proliferation and Well-Differentiated Adenocarcinoma of the Lung

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Background: Differentiating well-differentiated pulmonary adenocarcinoma, bronchioloalveolar carcinoma (BAC) and benign reactive bronchioloalveolar epithelium can be difficult on routine histology, especially with small biopsies. There is a limited available data regarding the utility of ancillary studies to help in this distinction. We investigated the staining pattern of commonly used the myoepithelial markers p63 (a p53-homologous nuclear protein), smooth myosin heavy chain (SMHC) and basal cell-specific cytokeratin antibody (34betaE12, K903) in benign and malignant bronchioloalveolar proliferations of the lung.

Design: We studied 60 lung lesions, consisting of 35 BAC, 30 well differentiated adenocarcinoma and 20 cases of benign lesion with fibrosis and metaplasia. Cases were immunostained for p63, SMHC and K903, using immunostaining autostainer with appropriate positive and negative control. Cases were reviewed and the pattern of staining was reported. The diagnostic categories of benign lung conditions were usual interstitial pneumonia, parenchymal scar, and cryptogenic organizing pneumonia.

Results: In normal lung, p63, SMHC and K903 decorated the reserve cells of large and small airways and occasional cells of the distal lobular unit. p63 intensely stained nuclei of bronchial reserve cells in all cases (100%) but did not stain ciliated cells, alveolar epithelial cells, or nonepithelial cells. In fibrotic reactive processes, a distinct pattern of nuclear staining was present in all cases (100%), with staining of basal cells of the airways as well as bronchiolar- and squamous-metaplastic epithelium for p63 and SMHC while 6/20 (30%) stained with K903. In BAC, a discontinuous peripheral rim of p63-immunoreactive cells was retained surrounding the malignant bronchioloalveolar proliferation in 31/35 (88.5%) cases, SMHC in 28/35 (80%) and K903 in 19/35 (54%) cases. Some cases showed positive cells intermingled with the negative ones. Adenocarcinomas cases were negative for myoepithelial markers, however; SMHC showed artifact staining for desmoplastic stroma in 6/30 (20%).

Conclusions: Our results highlighted the differential expression of p63 across various bronchioloalveolar lesions. Staining pattern of myoepithelial cells in BAC support that these neoplasms are actually carcinoma in situ. This would explain their good prognosis and their tendency not to metastases. Moreover, p63 may be helpful in distinguishing BAC from invasive adenocarcinoma of the lung in small lung biopsies.

1472 FGF-2 Expression Correlates with Tumor Grade in Pulmonary Neuroendocrine Neoplasms

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Background: Fibroblast growth factors (FGFs) are important regulators of processes determining cell fate and differentiation, proliferation, and angiogenesis, and activate the ERK1/2 MAP kinase pathway. In non-small cell lung cancers, FGF-2 and ERK 1/

2 expression have been linked to tumor metastasis, but little information is available about FGF-2 and ERK1/2 expression by pulmonary neuroendocrine tumors.
Design: Tissue microarrays were prepared from triplicate punch samples of 109 neuroendocrine lung tumors [53 carcinoids, 11 large cell neuroendocrine carcinomas (LCNECs), 45 small cell carcinomas (SCCs)] and immunostained for FGF-2 (1:100; Santa Cruz Biotechnology, Inc.) and ERK1/2 (1:50; Cell Signaling Technology). For each sample, tumor cell staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate) or 3+ (strong), and the percentage of tumor cells staining was assessed. Mean intensities and percentages were calculated for each tumor and used to provide an overall score between 0 and 3. Information about the presence or absence of metastasis was obtained for 30 subjects. Statistical analysis was performed using non-parametric methods.
Results: FGF-2 was expressed by most carcinoids (92.5%), LCNECs (90.9%) and SCCs (84.1%), while ERK1/2 was expressed by 37.7% of carcinoids, 9.1% of LCNECs, and 6.7% of SCCs. Metastasis was significantly associated with reduced percentages of tumor cells expressing FGF-2 (p=0.02). There was a significant difference in the average percentage of tumor cells expressing FGF-2 in the three tumor types (p=0.002), with carcinoids having highest expression (59.1±3.5, mean±SEM), LCNECs intermediate expression (49.8±6.8), and SCCs lowest expression (42.2±3.9). There was also a trend towards a decreased prevalence of metastasis with increased ERK1/2 score (p=0.07).
Conclusions: Higher levels of FGF-2 and ERK 1/2 expression are associated with lower grade pulmonary neuroendocrine tumors having a lower incidence of metastasis. ERK1/2 is expressed less commonly by neuroendocrine tumors than FGF-2, which probably reflects the influences of other regulatory molecules on ERK 1/2 expression.

1473 Alpha-Methylacyl CoA Racemase (AMACR) Expression in Pulmonary Carcinomas: A Study of 406 Cases

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Background: AMACR is an oxidative enzyme that is known to be overexpressed in prostatic carcinomas and serves as a useful diagnostic tool in genitourinary pathology. Very limited information is available in regard to its presence in pulmonary neoplasms, prompting us to investigate AMACR expression within the spectrum of pulmonary carcinomas, including neuroendocrine tumors (NET).
Design: Tissue microarray (TMA) based samples of 150 squamous cell carcinomas (SqCC), 150 adenocarcinomas (ADC), 51 typical carcinoids (TC), 25 atypical carcinoids (AC), 31 large cell neuroendocrine carcinomas (LCNEC), 69 small cell carcinomas (SmCC) and 50 normal (NR) pulmonary parenchyma cores from different patients were studied immunohistochemically with AMACR/p63 cocktail (Zeta). IHC results were recorded as positive or negative. Intensity of staining was scored as 1+ (weak), 2+ (moderate) or 3+ (strong). Disagreement on duplicate cores was negotiated by positive result/higher intensity overcoming negative/lower intensity or artifacts.
Results: Pulmonary tumors were positive with AMACR in 47.5% of cases: 23.1% of SqCC, 56.6% of ADC, 69.6% of TC, 47.8% of AC, 68% of LCNEC, and 51.6% of SmCC. 3+ tumors comprised 0% of SqCC, 4.7% of ADC, 0% of TC, 4.4% of AC, 8% of LCNEC and 12.9% of SmCC. All 5 NR bronchial columnar epithelium cores were positive with AMACR. NR pulmonary parenchyma did not show AMACR expression. AMACR IHC produced typical cytoplasmic microgranular pattern of staining throughout the whole spectrum of the tumors, while p63 decorated nuclei of SqCC.
Conclusions: Pulmonary carcinomas display AMACR expression in a significant percentage of cases. AMACR is more often expressed in ADC and NEC, than in SqCC. Given the high frequency of AMACR expression in pulmonary ADC, it is not useful in separating primary pulmonary versus metastatic prostatic carcinomas. High grade NEC (SmCC and LCNEC) tend to have higher levels of AMACR than intermediate grade (AC) or low grade (TC) tumors.

1474 Enhanced Pulmonary Expression of Endothelin-1 in Lung Tissue after Inhalation Injury

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Background: Endothelin-1(ET-1) has known as a contributer to the airway inflammation, mucus secretion, and pulmonary hypertension. This study was aimed to assess the immunohistochemical expression of ET-1 in the lung tissue following inhalation injury.
Design: ET-1 immunoreactivity was assessed in brochoscopic tissue specimens of normal lung(5) and lungs(56) at two weeks after inhalation injury, with histological evaluation for the status of epithelial alteration, mucus film, basement membrane, mucous gland alteration, inflammatory cell infiltration, pigment and soot deposition, and grading of fibrosis in lamina propria.
Results: In normal lung, ET-1 expression was limited to the basal epithelial portions of the large bronchi and mucous glands. In lung tissue after inhalation injury, ET-1 immunoreactivity was newly expressed in smaller bronchiolar epithelium, and in lamina propria, smooth muscle, and vessel walls, and not expressed in areas of inflammatory cell infiltration, pigment and soot deposition, and edema in lamina propria. This ET-1 expression strongly associates with the degree of fibrosis in lamina propria significantly(r=0.89).
Conclusions: ET-1 may contribute to increased airway fibrosis, causing airway resisitance and decreased compliance in lung tissue after inhalation injury.

ET-1 expression in 56 lungs after inhalation injury

Fibrosis grade	0(1)	1(1)	2(9)	3(45)
ET-1 expression 0(3)	1	0	0	2
1(12)	0	0	2	10
2(11)	0	0	2	9
3(30)	0	1	5	24

(numbers of lung)

1475 Immunohistochemical Analysis of Langerin in Langerhans Cell Histiocytosis and Other Pulmonary Inflammatory and Infectious Diseases

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Background: Pulmonary Langerhans cell histiocytosis (LCH) affects adult smokers and is histologically characterized by a nodular interstitial proliferation of Langerhans cells around distal airways with variable numbers of interstitial eosinophils, lymphocytes, fibroblasts, and pigmented alveolar macrophages. Immunostains for CD1a and S-100 have traditionally been used to distinguish LCH from other processes. Recently, an antibody directed against a Langerhans cell-specific lectin, termed langerin, has been developed. Little is known about the expression of langerin in pulmonary diseases. The purpose of this study was to evaluate langerin expression in LCH and other inflammatory and infectious pulmonary disorders, in comparison to S-100 and CD1a.
Design: 5 cases of LCH, 5 usual interstitial pneumonitis (UIP), 4 hypersensitivity pneumonitis (HP), 4 bronchiolitis obliterans and organizing pneumonia (BOOP), 4 sarcoidosis, and 5 viral, 2 fungal, and 3 mycobacterial pneumonias were immunostained for langerin, S-100 and CD1a. The extent of immunoreactivity was graded according to the number of cells staining per HPF.
Results: All cases of LCH showed strong langerin positivity (>100 per HPF) in lesional tissue, paralleling S-100 and CD1a expression. Characteristic langerin staining patterns emerged in other interstitial processes. Peribronchial langerin positivity was observed in all cases of UIP (mean 15; range 5-22), BOOP (mean 8; range 1-18) and HP (mean 8; range 3-20). Expression of CD1a, but not S-100, was similar to that of langerin in UIP, BOOP, and HP. In contrast, fungal and mycobacterial pneumonias showed strong S-100 staining adjacent to necrotizing granulomas (mean 21; range 7-50) without langerin or CD1a staining. Highly variable peribronchial S-100, langerin, and CD1a staining was seen in sarcoidosis. Finally, CMV pneumonitis was associated with a mild increase in S-100 positive dendritic cells (mean 10; range 3-21) with rare langerin and CD1a positive cells.
Conclusions: High density expression of langerin and CD1a is highly specific for LCH. In contrast, S-100 is less specific, since S-100-positive cells are markedly increased in some infectious disorders. Langerin and CD1a appear to be increased in several other interstitial diseases in a peribronchial distribution. This latter finding may be of pathogenetic importance and warrants further study. In conclusion, langerin is a useful diagnostic marker in distinguishing LCH from other pulmonary interstitial and granulomatous diseases.

1476 Evaluation of Cell Cycle Signaling Proteins ERK1-2 and Cyclin D-1 Expression in Non-Small Cell Carcinoma (NSCLC) and Correlation with Survival

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Background: ERK 1-2 and Cyclin D-1 are signaling proteins that have a significant role in regulation of cell proliferation, differentiation and adaptation. A recent report links cytoplasmic staining by ERK 1-2 to advanced tumor stage in NSCLC. Overexpression of Cyclin D-1 is believed to cause cell cycle arrest and increase apoptosis. We evaluated ERK 1-2 and Cyclin D-1 in 344 NSCLC with long term clinical follow-up.
Design: Tissue microarrays (TMA) were prepared from triplicate 1mm sample punches from 344 NSCLC and stained for ERK 1-2 (1:50 Cell Signaling, Beverly, MA) and Cyclin D-1 (1:300 Biocare, Concord, CA) using standard immunohistochemistry techniques. Percent of cytoplasmic and/or nuclear staining was evaluated and value averaged for each tumor. Staining intensity was scored from 0-3 (0 = negative, 1 = weak, 2 = moderate and 3 = strong) for each punch and an average value calculated for each tumor. Information about tumor stage and survival was available. Results were analyzed using Kaplan-Meir analysis.
Results: The average patient age was 65 yrs. with an average follow-up of 50.3 months. Of the 344 NSCLC, 54% were adenocarcinomas, 26% were squamous cell carcinomas and 20% were large cell. 65% were Stage I tumors and 16% Stage II. The tumor cells showed nuclear staining for Cyclin D-1 and both cytoplasmic and nuclear staining for ERK 1-2 with a majority of the cells showing cytoplasmic staining. Cyclin D-1 intensity of staining (+2,+3) and decreased ERK 1-2 (<30%) staining showed a trend to better survival but no significant association with stage, cell type or grade. Only squamous cell carcinomas with > 40% percentage staining with ERK 1-2 showed an impact on survival (p = 0.02). Expression of ERK 1-2 and Cyclin D-1 were not significantly correlated.
Conclusions: In early stage squamous cell carcinoma there appears to be a significant association between increased ERK 1-2 expression and poor survival. No significant association between survival and expression of ERK 1-2 was found in adenocarcinomas. Tumors with overexpression of Cyclin D-1 showed a trend towards longer survival. These results suggest possible new treatments related to modulation of cell cycle related proteins in early stage squamous cell carcinoma.

1477 The Movat Pentachrome Stain Facilitates the Recognition of Microcrystalline Cellulose among Other Crystals Found in Lung Tissue

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Background: Microcrystalline cellulose (MCC) is a birefringent fibrous cellulose compound extensively used as a filler material in pharmaceutical tablets. When injected intravenously as a component of an aqueous tablet suspension, MCC may contribute to obliterative embolic pulmonary hypertension. In this study we evaluated the Movat Pentachrome Stain (MPS), traditionally used to highlight elements of connective tissue, as a means of identifying MCC and distinguishing it from other birefringent crystals such as talc, mixed silicates, and calcium oxalate in lung tissue specimens.

Design: Archival, formalin-fixed, paraffin-embedded autopsy or surgical pathology lung specimens containing various birefringent crystals, including MCC (3 cases of intravenous drug abuse [IVDA]) , talc (2 cases of IVDA, 1 talc pleurodesis), mixed silicates (1 case of silicate pneumoconiosis), inhaled cellulose fibers (1 case), and calcium oxalate (1 case of aspergillosis niger), were respectively evaluated with the MPS. Crystal identification was confirmed by morphology, other histochemical stains, infrared spectroscopy (1 case), and known cellulose controls.
Results: MPS consistently stained MCC and inhaled cellulose fibers intensely bright yellow in tissue and control specimens. Talc was tinted light greenish-blue, while mixed silicates appeared either greenish-blue or unstained. Oxalate crystals were digested from the tissue by the acetic and phosphotungstic acid steps of the MPS procedure and were therefore unstainable by MPS. Crospovidone, a non-birefringent amorphous tablet filler substance (also present in 3 of the IVDA specimens), stained yellow green with MPS and was easily distinguished from MCC. Starch granules, present in a control specimen were unstained by MPS.
Conclusions: In addition to its value in studying the pulmonary vasculature and interstitium, MPS is an excellent method for the histochemical identification of MCC in tissue and its separation from other birefringent crystals with which MCC might be confused. MPS is especially useful in the evaluation of pulmonary foreign body embolization in cases of suspected intravenous substance abuse.

STAINING OF PARTICLES BY MPS

Particle	Result
Microcrystalline Cellulose	Bright yellow
Talc	Light green-blue
Mixed silicates	Light green-blue or unstained
Oxalate	Digested from tissue
Starch	Unstained
Crospovidone	Yellow-green

1478 Molecular Evaluation of Multiple Squamous Cell Carcinomas of the Head and Neck and Lung: Metastasis vs. Separate Primaries

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Background: The lung is the most common site for metastases of head and neck squamous cell carcinomas (SCC). However, in patients that have had a head and neck SCC, the SCC of the lung could also represent a pulmonary primary, since both tumors are associated with smoking and have a similar morphologic appearance. The distinction between a second primary and a metastasis can have prognostic and therapeutic implications, but histologic examination can't accurately make this diagnosis. In this study, we evaluated the efficacy of molecular analysis for this distinction.
Design: Paraffin blocks were retrieved from 24 cases of 12 patients. 4-8 microdissection targets from neoplasm and non-neoplastic controls were removed under stereoscopic guidance and analized by PCR for LOH at 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q and point mutation determination in k-ras-2 . These markers were chosen based on molecular abnormalities reported in the literature and on preliminary data from our laboratory. The tumors were classified as de novo or metastasis based on 3 levels of concordance: (1) marker affected - tumors were considered concordant if 50% or more of the same markers were mutated, (2) same gene copy affected, and (3) temporal sequence of mutation. We reviewed the H and E slides to determine by histology if we favored metastases or separate primaries based on several criteria: differentiation, keratinization, special type, presence of in situ carcinoma, and subjective impression of the pathologist.
Results: Molecular analysis demonstrated that 4/12 of the lung SCCs represent metastases from the head and neck primary and 8/12 SCCs represent de novo carcinomas. The comparison of morphologic and molecular evaluation is shown in the table below:

	De novo	Metatasis
Similar by morphology	3	2
Different by morphology	5	2

Conclusions: We believe that the molecular analysis by identifying multiple concordant molecular abnormalities can determine if a SCC involving the lung in a patient with a previous or concurrent head and neck SCC represents a de novo lung carcinoma or a metastasis. There was poor correlation between histology prediction versus molecular profiling, reflecting limitations of microscopy. Molecular profiling including temporal pattern of mutation acquisition provides the means to determine the nature of the lung SCC in patients with SCC of the head and neck.

1479 Cellular Localization of b-Catenin Correlates with Expression of Proliferation Markers in Diffuse Malignant Mesothelioma (DMM)

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Background: The adhesion molecule b-catenin is a key participant in the Wnt signaling pathway, implicated in the pathogenesis of many cancers. The Wnt signaling cascade results in expression of proliferative markers, C-myc, cyclin D1, and MMP-7. It has been suggested that dysregulation of this pathway may lead to accumulation of b-catenin in the cytoplasm and ultimately the nucleus, where Wnt target genes are activated. Increased β-catenin levels may trigger cyclin D1 gene expression and uncontrolled progression into the cell cycle causing neoplastic progression. We studied b-catenin expression in DMM.
Design: 43 surgically sampled formalin-fixed, paraffin-embedded DMM were used to create a tissue microarray containing three representative samples from each tumor. Recut sections were immunostained for b-catenin (1:300, BD Transduction), c-myc (1:3000, Abcam), cyclin D1 (BioCare, 1:300), and MMP-7 (Abcam, 1:50) and the Envision + Labeled Polymer Universal Kit (DakoCytomation, Carpenteria CA). Staining intensity was scored as negative, weak, moderate, or strong, and percent positivity was estimated to the nearest 10%. ANOVA and Spearman rank correlation tests were performed to establish relationships among the data.
Results: 36 of the 43 (84%) cases showed expression of b-catenin. In 12 cases (28%) nuclear localization of b-catenin was present. 100% of tumors expressed c-myc, 75% expressed cyclin D1, and 68% expressed MMP-7. C-myc expression correlated with nuclear (r = 0.38) and cytoplasmic b-catenin (r = 0.52). Cyclin D1 expression correlated with cytoplasmic b-catenin (r = 0.52). MMP-7 expression correlated with nuclear (r = 0.39) and cytoplasmic b-catenin (r = 0.50).
Conclusions: b-catenin is expressed by most DMM with abnormal nuclear localization of b-catenin in 28%. Nuclear localization of b-catenin correlates with expression of c-myc and MMP-7. Cytoplasmic localization of b-catenin correlates with expression of c-myc, cyclin D1, and MMP-7. These observations suggest that abnormal localization of b-catenin may lead to elevated expression of specific proliferation markers and may play a role in the pathogenesis of DMM.

1480 Immunoreactivity of Anti-L523S on Normal and Malignant Lung Pleural Tissue Biopsies

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Background: The positive identification of malignant cells in lung pleural tissue biopsies can be challenging, particularly when normal and reactive mesothelial cells are present. L523S, also known as K homology domain containing protein overexpressed in cancer (KOC), is a 580 amino acid oncofetal RNA binding protein that regulates insulin-like growth factor II (IGF-II) transcripts during embryogenesis. It is later re-expressed in a proportion of neoplastic cells from various types of tumors, including pancreatic and lung adenocarcinoma. L523S mRNA expression has been found to be minimal in many normal tissues using RT-PCR and microarray analysis. In this study we evaluated the expression of L523S in normal and reactive mesothelial cells and in malignant cells of lung pleura.
Design: Formalin fixed paraffin embedded (FFPE) normal lung pleural tissue biopsies and lung pleural biopsies containing: mesothelioma, melanoma, adenocarcinoma, spindle cell carcinoma, and squamous cell carcinoma were obtained. Normal pericardium tissue with an intact mesothelial cell layer was also procured. The sectioned slides were pretreated with heat-induced epitope retrieval using a Tris/EDTA target retrieval solution. Immunohistochemistry was performed with mouse monoclonal antibodies to human calretinin and/or L523S. Bound antigen was detected using the DakoCytomation Envision + Detection System.
Results: Normal lung pleural tissue biopsy samples contained mesothelial cells, as indicated by positive Calretinin staining. Anti-L523S was unreactive with all of these normal pleural mesothelial cells. The majority of the lung pleural biopsy samples containing mesothelioma demonstrated L523S expression. In addition, lung pleural biopsies containing: melanoma, adenocarcinoma, spindle cell carcinoma, and squamous cell carcinoma were also positive for L523S protein expression. Normal pericardial mesothelial cells were found to positively immunostain with anti-L523S, differing form L523S negative normal pleural mesothelial cells. Very faint L523S immunoreactivity was also seen within a subset of lung bronchial cells.
Conclusions: Anti-L523S may have utility as a marker of malignancy in biopsy samples of lung pleura. L523S protein was not expressed in the normal pleural mesothelial cells tested, but was found in the majority of cancers evaluated within lung pleural biopsies, including mesothelioma. While L523S is not specific for mesothelioma, its expression can aid in differentiating benign from malignant pleural mesothelial cells.

1481 Malignant Mesothelioma (MM) Represents a Single Epidemiologic-Pathologic Population Despite Its Histologic Variants

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Background: Malignant Mesothelioma of the pleura presents with protean histopathologic patterns. We have investigated age-specific incidence rates and age-density distributions, using graphical analysis, to assess possible carcinogenic homogeneity for MM and to propose a pathologic mechanism for its development.
Design: Age-specific incidence rates were obtained from MM cases reported to the SEER Program of the NCI for the years 1992 through 2002. Age-specific incidence rate patterns (log-log scale), density distributions, and hazard functions were plotted for the different morphological variants of MM and for their gender distributions.
Results: Graphical analysis of the age-specific rates for the variants of MM identified a family of curves with similar plots that differed markedly from those tumors that are considered within the differential diagnosis of MM. Density plots and hazard analysis for survival also identified a single tumor population for MM, different from other pleural-based malignancies. Slopes of logarithmic age-specific incident rate plots are identical for men and women.
Conclusions: Age-specific incidence rates and density distributions suggest that mesotheliomas represent a single population of tumors despite their histologic variants, are epidemiologically separate from other tumors having similar histopathologic patterns, share a common carcinogenic pathway, and that, epidemiologically and pathologically, mesotheliomas are identical in men and women, suggesting the absence of hormonal modulation.

1482 Evaluation of Alpha-methyacyl-coenzymeA racemase(AMACR) Expression in Pulmonary Bronchioloalveolar Adenocarcinomas

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Background: Mucinous bronchioloalveolar carcinomas immunophenotypically differ from conventional pulmonary adenocarinomas and occasionally need to be differentiated from metastatic mucinous colonic adenocarcinomas. Both mucinous bronchioloalveolar carcinomas and metastatic mucinous colonic adenocarcinomas express cytokeratin 20,

which can be a diagnostic challenge. AMACR, a mitochondrial and peroxisomal enzyme, involved in beta-oxidation of fatty acids is a sensitive marker for prostate cancer. It is reportedly variably expressed in pulmonary non-small cell carcinomas. Strong expression of AMACR has been reported in majority of colorectal adenocarcinomas. We studied the expression of AMACR in bronchioloalveolar carcinomas (BAC) to analyze its utility as a differential marker for identifying BACs.

Design: A 2.0 mm core tissue microarray(TMA) of 32 cases was created, comprising 11 mucinous BACs(mBAC), 7 nonmucinous BACs(nmBAC) and 14 pulmonary adenocarcinomas with focal bronchioloalveolar features. TMA sections were stained for AMACR using a monoclonal antibody (P504S, Zeta, Sierra Madre, CA). Tumor cell staining was scored for intensity of staining as - 0(negative staining), 1+(weak staining), 2+(moderate staining) and 3+(strong staining). Immunoreactivity was considered negative in cases with weak staining of <5% of cells. Positivity was divided into focal (5-50% positive cells) or diffuse (>50% positive cells).

Results: None of the cases of BAC stained positive for AMACR, while 1/14 cases of pulmonary adenocarcinoma showed weak focal staining in the adenocarcinoma tumor cells.

Conclusions: These results indicate that BACs are negative for AMACR antibody. Though AMACR antibody may not be an independent immunomarker for identifying BACs; preliminary evidence here suggests that it may be a useful negative biomarker in an immunopanel to differentiate from metastatic colonic adenocarcinomas.

	Positive	Negative	Total
Mucinous BAC	0	11	11
Nonmucinous BAC	0	7	7
Pulmonary adenocarcinoma	1	13	14
			32

1483 XIAP Immunostaining Distinguishes Malignant Mesothelioma from Benign Mesothelial Hyperplasia

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Background: The diagnosis of mesothelioma is currently based on morphology; there is no immunomarker to distinguish it from benign reactive or hyperplastic mesothelium. One feature that distinguishes many cancer cells from their normal counterparts is the aberrant activation of pathways that promote survival and/or suppress apoptosis. We have previously surveyed X-linked inhibitor of apoptosis (XIAP), a potent constituent of the inhibitor of apoptosis (IAP) family of proteins in cytology cell block samples of body cavity effusions. Increased XIAP expression was found in many malignancies, including 4 mesotheliomas.

Design: Material and methods: Tissue sections (13 malignant mesothelioma and 12 mesothelial hyperplasia) from formalin-fixed paraffin embedded surgical tissue blocks were subjected to citrate based antigen retrieval then incubated with monoclonal anti-XIAP (# 610763, BD Biosciences, San Jose, USA) 1:250, 4°C for 72 h and developed using EnVision-Plus reagents (Dako) and diaminobenzidine as chromagen. Particulate cytoplasmic staining was considered positive.

Results: Twelve of 13 (92.3%) malignant mesothelioma cases displayed moderate to strong XIAP positivity. All cases of benign mesothelial hyperplasia were virtually XIAP-negative except for weak nonspecific staining sometimes noted in inflammatory cells or histiocytes.

Conclusions: XIAP immunostaining, when strong, allows for ready distinction of malignant from benign and reactive mesothelial cell populations and is a potentially valuable immuno diagnostic marker in both surgical and cytological samples. Elevated expression of XIAP could contribute to tumorigenesis in mesothelioma. Acknowledgement: This work was supported by generous bequest from the Estate of Hilda Leven (DEB).

1484 Quantitation of Lymphocytes in Lung Allograft Biopsies with and without Rejection

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Background: Cellular allograft rejection in lung biopsies is based on inflammation in two compartments: bronchial mucosa and alveolar walls. We quantitated lymphocytes in both compartments in a series of lung transplant biopsies to determine if immunohistochemical lymphocyte phenotype is useful in grading transplant rejection.

Design: Thirty-five lung transplant biopsies from 20 lung allograft recipients were studied immunohistochemically for CD3, CD8, CD1a and CD57, and dendritic cells (S-100 protein). Numbers of positive cells were quantitated per mm2 in the alveolar spaces, with care taken to avoid areas of collapse. Lymphocytes were quantitated in bronchial epithelium per 100 epithelial cells. Nine biopsies from controls without significant lung disease were studied similarly. In the transplant biopsies, cell density was correlated with indication for biopsy (surveillance [n=10]; presumed rejection [n=11}; presumed infection, determined clinically and biopsy findings [n=8]; and other causes for decreased lung function [n=6]). Rejection was graded as A0-A4, B0-B4, and cell density correlated with grading of rejection.

Results: There were few CD1a, CD57 and S-100 positive cells in any of the groups. CD3 positive parenchymal cells were found at the following density: 39.6 ± 5.3 controls, 57.5 ± 15.0 surveillance, 78.0 ± 18.0, miscellaneous non-rejection lung disease, 102.4 ± 30.0 infection, 262.1±137.4 suspected rejection (p= .03 control vs. suspected rejection, p= >0.2, control vs. surveillance). CD3 parenchymal staining, by grading of rejection based on routine stains showed: A0: 50.2±14.2; A1: 120.8 ±40.9; A2: 134.3± 21.7 (p<.01 A0 vs. A2). CD8 positive parenchymal cells were found at the following density: 47.7 ± 5.3 controls, 65.3 ± 13.1 surveillance, 64.0 ± 14.6, miscellaneous non-rejection lung disease, 109.8 ± 35.1 infection, 204.1 ± 95.8 suspected rejection (p= .03 control vs. suspected rejection, p= >0.2, control vs. surveillance). Grading of rejection based on routine stains showed the following CD8 densities: 58.6 ± 18.5 A0; 101.0 ± 21.0 A1; 146.9 ± 10.3 A2 (p=.03 A0 vs., A2). CD3 positive bronchial intraepithelial lymphocytes averaged 5.0 ± 1.3 in B0, 7.1 ± 2.8 in B1, and 15.7 ± 4.0 in B2 (P<.01, B2 vs. B0). Corresponding values for CD8 were: 5.1 ± 1.3 in B0, 8.8 ± 2.8 in B1, and 15.4±3.9 in B2 (p<.01, B0 vs. B2).

Conclusions: Quantitation of T-lymphocytes, both CD3 and CD8 subsets, may be helpful in grading cellular rejection in lung allograft biopsies.

1485 Neuroendocrine (NE) Lung Tumors: A Clinicopathologic Study of 515 Cases

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Background: NE tumors of the lung consist of low grade typical carcinoid (TC), intermediate grade atypical carcinoid (AC), and high grade large cell NE carcinoma (LCNEC) and small cell carcinoma (SCLC). AC and LCNEC are so rare little is known about their biologic behavior and therapy. This study was undertaken to determine the clinical characteristics of these tumors.

Design: 515 NE tumors of the lung were reviewed from the institutions of the authors. Clinical data was collected from medical records and followup was obtained from tumor registries, consultation files, hospital records and social security death index. The tumors were classified according to the 2004 WHO Classification. Statistics were performed with SPSS v 13.

Results: 92 TC, 128 AC, 154 LCNEC and 141 SCLC were found with a mean age (range) of 51 (18-82), 55 (9-92), 63 (33-87), 64 (30-87) yrs, respectively. The mean age was significantly older for AC compared to TC (p=0.040) and for LCNEC and SCLC compared to AC (p<0.001). M:F ratios were similar in TC (47:45) and AC (66:62) with a strong male predominance in LCNEC (111:43) and SCLC (97:44)(p<0.001). The 5 year survival was 97% for TC, 52% for AC, 16% for LCNEC and 12% for SCLC (p<0.001). Stratified for stage 5- yr survival was significantly worse for AC than TC (p<0.001) and for both LCNEC and SCLC compared to AC (p<0.001). 5-yr survival for Stage 1, 2 and 3 TC was 98%, 100% and 67%; for Stage 1, 2, 3 and 4 AC was 74%, 34%, 52% and 12%; for Stage 1, 2, 3, and 4 LCNEC was 25%, 0%, 27%, and 1% and for Stage 1, 2, 3 and 4 SCLC was 20%, 0%, 9% and 0. There was no significant difference in survival between LCNEC and SCLC. Data regarding adjunctive therapy was insufficient to determine effectiveness for AC and LCNEC.

Conclusions: Even with Stage 2 or 3 disease, patients with TC have an excellent survival. Survival for AC is significantly better than that for LCNEC and SCLC. Due to the rarity of AC and LCNEC, even with this number of cases there is insufficient data on how to treat these patients so an international registry is proposed to characterize these tumors and develop a uniform approach to treatment and monitoring responses.

1486 Is There an Idiopathic Nonspecific Interstitial Pneumonia (NSIP)?

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Background: Nonspecific interstitial pneumonia (NSIP) describes a pulmonary disorder characterized by a uniform interstitial process with a lymphoplasmacytic infiltrate and varying degrees of fibrosis. Like other categories of interstitial pneumonitis, NSIP is held to occur in response to a variety of conditions, as well as an idiopathic entity. While poorly understood, the latter's significance is largely its difference from another idiopathic chronic pneumonitis, usual interstitial pneumonia (UIP).

Design: To better define idiopathic NSIP and the conditions that underlie secondary NSIP, a retrospective clinico-pathological review was conducted of all open lung biopsies from 1995 to 2005 that meet the criteria for NSIP. Included cases (75) evidenced a temporally uniform, homogeneous interstitial pneumonitis with inflammation and/or fibrosis that did not display the diagnostic features of other entities.

Results: Twenty eight cases (37%) were iatrogenic in origin, secondary to drug toxicity (10), radiation (4), or bone marrow transplantation (14). The latter included the toxicity of chemotherapy (6), the idiopathic pneumonia syndrome (5), and pulmonary graft-versus host disease (3). A 2nd group (19) comprised those with an immunologic basis, an autoimmune or collagen vascular disease (CVD) (11), hypersensitivity pneumonitis (5), or hepatitis C with cryoglobulinemic alveolitis (3). Chronic passive congestion was the cause in 9 (12%). In 5 (7%) smoking caused a pneumonitis that was neither typical desquamative interstitial pneumonitis nor respiratory bronchiolitis-interstitial lung disease. One patient inhaled a toxic chemical, and 1 case represented a resolving pneumonia. The last group (12 or 16%) was of unknown etiology, including 1 likely idiopathic BOOP. Four had NSIP in association with a chronic bronchiolitis (CB). Six averaged 22.6 years, were asthenic (mean BMI 20.1 kg/m^2), and presented with a spontaneous pneumothorax (SP). NSIP and associated pleural fibrosis likely caused the SP. Only 1 was considered for an associated CVD. Finally, 1 patient corresponded to what has been termed idiopathic NSIP, a 45 year-old woman who came to the hospital for biopsy and was discharged without any diagnostic workup or subsequent follow-up.

Conclusions: In all but 1 of the 75 patients, a cause of the NSIP could be assumed (64 cases) or an associated condition (CB or SP) identified (10 cases). Thus, in the present experience, truly idiopathic NSIP is a rare disorder that may exist only in the absence of a thorough clinical evaluation.

1487 Over-Expression of Aspartyl (asparaginyl) β-Hydroxylase in Lung Cancer

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Background: Over-expression of aspartyl (asparaginyl) β-hydroxylase (AAH), a dioxygenase that catalyzes asparaginyl residues present in epidermal growth factor like domains of certain proteins, has been demonstrated in various malignant neoplasms including liver and pancreas. Recent studies demonstrated that AAH has an important role in regulating invasive and metastatic tumor cell growth and, that high levels of AAH expression correlate with aggressive histology and poor clinical prognosis. The present study characterizes the expression of AAH in different sub-types of lung cancer.
Design: Histological sections and tissue microarrays were created from archival paraffin embedded tissue samples from 90 patients with lung carcinomas, including 38 adenocarcinomas (18 adenocarcinomas, NOS; 14 bronchioloalveolar carcinomas and 6 with papillary features); 21 squamous cell carcinomas; 17 small cell carcinomas; 11 undifferentiated and 3 large cell neuroendocrine carcinomas. The sections were stained with the FB-50 monoclonal antibody to AAH protein.
Results: 67% of the neoplasms exhibited AAH immunoreactivity: 40% with moderate to intense levels of staining and 27% with weak reactivity; 33% were negative. Well differentiated adenocarcinomas (83% of adenocarcinomas with papillary features and 72% of bronchioloalveolar carcinomas) exhibited intense staining; whereas, 41% of adenocarcinomas (NOS), 36% of undifferentiated carcinomas, and 43% of squamous cell carcinomas had intermediate levels of immunoreactivity; large cell neuroendocrine and small cell carcinomas had weak to negative levels of staining. A statistically significant relationship between the intensity of AAH immunoreactivity in the three levels of immunoreactivity was seen (low & high $p<0.0001$; intermediate & high $p=0.001$; and low & intermediate $p=0.01$) .
Conclusions: AAH immunoreactivity is detectable in 67% of lung carcinomas, consistent with previous reports detecting over-expression of the corresponding AAH gene in other non-pulmonary malignant neoplasms. Interestingly, stronger AAH stain was seen in well differentiated adenocarcinoma. This is in contrast to what was been reported in other malignancies in which AAH expression was strongest in higher grade tumors. Additional studies are ongoing to determine the prognostic significance of AAH expression in lung carcinoma.

1488 Comparison of Treatment Effect in Non-Small Cell Lung Cancer Treated with High Versus Low Energy Photons

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Background: Most cooperative group trials involving radiation for lung cancer use treatment with low photon energies ($\leq$ 6MV). When treating with higher energy photons, most radiation treatment planning systems are unable to accurately determine the dose at the interface of the low density lung and higher density tumor, potentially leading to poorer response. Our approach has involved the use of higher energy photons ($\geq$15MV) in the neoadjuvant setting due to better dose homogeneity which could result in decreased postoperative mortality and morbidity. To the best of our knowledge, there has been no studies comparing pathologic tumor response to these regimens.
Design: 27 patients with stage III non-small cell lung carcinoma were treated with 3 neoadjuvant radiation regimens: 11 were treated with high energy, 6 with low energy, and 10 with mixed regimen. The diagnoses were as follows: 12 adenocarcinomas, 11 non-small carcinomas, and 4 squamous cell carcinomas; the tumor types were represented equally in treatment groups. Morphologic features of treatment effect in tumors and non-neoplastic lung tissue were evaluated by two observers who were blinded to the treatment protocol used in each case.
Results: There was no significant difference in pathologic response between the low and high energy regimens in the amount of residual viable tumor or tumor necrosis.Tumors treated with low energy photons showed more necrosis compared to mixed regimen (44.2% vs 17.7%), and least mucin lakes in adenocarcinomas (1.6% vs. 11.3%), $p<0.05$. Tumors treated with high energy photons had more fibrosis compared to mixed regimen (51.8% vs. 31%), $p<0.05$. Compared with low and high energy protocols, tumors treated with mixed regimen showed more residual tumor, 27.5% vs. 9.3% and 9.18 %, $p=0.02$; less necrosis, 17.7% vs. 44.2% and 28.2%, $p<0.05$; thinner fibrous border around the tumor (0.28cm vs. 0.39 and 0.35 cm, $p=0.03$), and more extensive radiation effect in non-neoplastic lung: more common diffuse alveolar damage pattern (40% vs. 16% and 18%) and edema (40% vs. 0 and 27%).
Conclusions: Contrary to predictive models in radiation oncology, there was no major difference in tumor or normal tissue response between low energy and high energy regimens. The mixed regimen showed the least tumor response and most tissue damage in the benign lung. This study is ongoing and its results could have implications for the design of cooperative group trials and in the treatment of lung cancer in general.

1489 Detection of EGFR and HER2 Activating Mutations in Lung Adenocarcinoma by High Resolution Melting Amplicon Analysis

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Background: Recently, a subset of patients with non-small cell lung cancer (NSCLC) has been found who respond to the anticancer drugs gefitinb (Iressa) and erlotinib (Tarceva). Gefitinb and erlotinib are small molecule, quinazolinamine derivatives, which target the active site of the epidermal growth factor receptor 1 (EGFR). Many patients who respond to these drugs have been found to contain tumors with activating EGFR mutations. It appears from multiple clinical studies that one of the best predictors of response to gefitinib or erlotinib in patients with NSCLC is the presence of an underlying EGFR somatic mutation in the tumor. In this study we used High Resolution Melting Amplicon Analysis (HRMAA) to screen for activating mutations in EGFR and HER2 in lung adenocarcinomas.
Design: Thirty-nine patients with adenocarcinoma of the lung were identified by searching the surgical pathology archives at the University of Utah Health Sciences Center. All cases were primary lung adenocarcinomas. Twenty cases showed bronchioloalveolar carcinoma (BAC) features and 19 cases did not. Specifc primers were designed to amplify EGFR exons 18, 19, 20, 21 and HER2 exons 19, 20. These are exons where known activating mutations occur. After polymerase chain reaction (PCR) with specific primers, the PCR products were screened for activating mutations by HRMAA. All cases were followed up by bi-directional DNA sequencing. Fluorescence in situ hybridization (FISH) for EGFR and HER2 and expression of EGFR and HER2 estimated by immunohistochemistry (IHC) was performed on all cases.
Results: Six (15%) of the 39 patients had tumors which contained EGFR activating mutations. Four of the mutations were in adenocarcinomas which had some BAC features and 2 mutations were in tumors without BAC features. The EGFR mutations were in exon 19 (2 cases), exon 20 (2 cases) and exon 21 (1 case). One case contained mutations in both exons 18 and 20. Two of the 6 EGFR mutation positive cases showed polysomy for chromosome 7 and each one showed overexpression of EGFR as determined by immunohistochemical staining. The other EGFR mutation positive cases did not show EGFR overexpression and appeared disomic for chromosome 7. One (2.6%) of the 39 patients had a tumor with BAC features which contained a HER2 activating mutation (exon 20). This tumor did not show overexpression of HER2 and was disomic for chromosome 17.
Conclusions: HRMAA is able to detect EGFR and HER2 activating mutations in lung adenocarcinoma.

1490 Immunohistochemical Detection of XIAP and p63 in Adenomatous Hyperplasia, Atypical Adenomatous Hyperplasia, Bronchioloalvelolar Carcinoma and Well-Differentiated Adenocarcinoma

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Background: The distinction of bronchioloalvelolar carcinoma (BAC) and well-differentiated adenocarcinoma (WDAC) of lung from adenomatous hyperplasia (AH), and atypical adenomatous hyperplasia (AAH), is critical for patient management but can be very difficult. We examined expression of two markers, X-linked inhibitor of apoptosis (XIAP), the most potent inhibitor of the apoptosis (IAP) protein family and p63, a marker for reserve cells and squamous cells, in these entities.
Design: H&E slides of 37 tissue blocks from 27 patients were reviewed and classified as AH (n=8), AAH (n=11), BAC (n=9) and WDAC (n=13). Immunostaining was performed on 5 micron sections with monoclonal anti-XIAP (#610763, BD Biosciences, San Jose), 1: 250, 4^{o}C x72 hrs following citrate-based antigen retrieval, and developed using EnVision-Plus (Dako) and diaminobenzidine as chromagen; and monoclonal anti-p63 (4A4, Santa Cruz) diluted 1:4000 in PBS with 0.1% bovine serum albumin and 5% non-fat dry milk for 2 hrs at 37^{o}C. Granular cytoplasmic staining for XIAP and nuclear staining for p63 were considered positive.
Results: Both XIAP and p63 were negative in normal lung tissue except p63 positivity in bronchial reserve cells (BRC) and in some cases, XIAP apical positivity in bronchial cells. All AHs were negative for XIAP. 7/8 AHs were negative to focally positive for p63; 1 was strongly positive for p63. All AAHs were immuno-positive for XIAP (8/11 moderate; 3/11 focal to weak) and displayed rare positivity in 8/11 cases but diffusely strong in 3/11 cases in BRC for p63. All BACs displayed focal weak to diffuse strong XIAP positivity; p63 was negative in 7 out of 9 and focally positive in 2 out of 9. 12 out of 13 WDACs showed XIAP staining in a similar pattern to BAC and were negative for p63. One "WDAC" was negative for XIAP but strongly positive for p63.
Conclusions: Significant XIAP expression appears to be useful for distinguishing AAH from AH. Commonality of XIAP staining in AHH, BAC and WDAC supports the possibility that AAH may be a pre-malignant lesion. Rarity of expression of p63 confirms previous reports and supports a non-squamous histogenesis. In contrast, diffuse and strong p63 staining may facilitate the identification of rare cases that may have been misclassified as alveolar in origin based on morphology but are in fact either nodule-like proliferations or occasionally neoplasms of bronchial origin.

1491 Immunohistochemical Analysis of Potential Cell Signaling Biomarkers for Mutant Epidermal Growth Factor Receptor

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Background: Recent studies have shown that mutations in the Epidermal Growth Factor Receptor (EGFR) may be responsible for some patient sensitivity to EGFR kinase inhibitors such as Iressa and Tarceva; therefore, potential cell signaling biomarkers for mutant EGFR would be clinically useful.
Design: Using immunohistochemical (IHC) and tissue microarray(TMA) techniques, we studied EGFR phosphorylation and its downstream molecules in five human Non-small Cell Lung Cancer (NSCLC) xenograft models and 228 NSCLC patient samples

and compared EGFR and Akt pathway activation with EGFR mutation status. Slides were immunostained with activation-state specific antibodies, including antibodies to phospho-EGFR, phospho-tyrosine and phospho-Akt.12 cases were subjected to EGFR DNA sequencing.

Results: High EGFR phosphorylation of all the tyrosine sites probed and phosphorylated Akt were detected in mutant EGFR cell xenografts but not in xenografts containing cell lines with wild type EGFR. IHC analysis of general phospho-tyrosine, phospho-EGFR and phospho-Akt on a NSCLC TMA found phosphorylated EGFR in 15%-25% of patient samples, depending on the tyrosine site. Phosphorylated EGFR was more frequent in adenocarcinomas (20%-30%) and in bronchoalveolar carcinomas (BAC)(28%-50%) than in squamous cell carcinomas (SCC)(5%-10%). The phospho-EGFR reactivity was closely correlated with high general phospho-tyrosine reactivity. There was also a strong correlation between high phospho-EGFR staining and high phospho-Akt staining in patient specimens. EGFR kinase domain DNA sequencing revealed that a majority of these samples were mutant.

Conclusions: These results suggest that mutant EGFR is constitutively phosphorylated and activates downstream Akt in NSCLC; therefore, IHC analysis of phosphorylated EGFR and Akt may reflect the activation of mutant EGFR signaling in patient samples.

1492 Adhesion Molecules (α-Catenin, β-Catenin, and E-Cadherin), and EGFR Expression in Thymic Neoplasms with Clinicopathologic Correlation

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Background: To investigate the expression of α-catenin, β-catenin, E-cadherin and EGFR in thymic neoplasms, and analyze their interrelationship with cliniopathological variables and their effect on prognosis.

Design: A series of 61 thymic neoplasms were reviewed and classified according to World Health Organization (WHO) scheme. Key clinical information including patient survival, Masaoka's staging, disease local recurrence, treatment modality, and WHO histology subtype was obtained. Percentage of membranous expression of α-catenin, β-catenin, E-cadherin and EGFR were recorded. While staining <80% of the cells was considered reduced expression for α-catenin, β-catenin, and E-cadherin, EGFR was considered positive when >10% of tumor cells expressed the antibody. Correlation of expression of markers and clinicopathologic variables was statistically analyzed using Spearman rank correlation and Kaplan-Meier analysis.

Results: Fifty seven patients had available long follow up records. There were 6 type A, 15 type AB, 7 type B1, 6 type B2, 17 type B3, and 9 type C tumors. Six of fifty seven patients died of the disease. Six patients developed local relapse. The reduced expression rate of α-catenin, β-catenin, E-cadherin was 77.2%, 78.9%, and 89.5% respectively in all subtypes. Expression of α-catenin was significantly reduced (<30%) in patients who died of the disease (p=0.04). Expression of β-catenin closely correlated with α-catenin, and E-cadherin (p<0.001 each). Epidermal growth factor receptor was positive in 56.1% of total cases with no significant correlation with various prognostic variables.

Conclusions: The reduced expression of α-catenin, but not β-catenin, and E-cadherin, implies more aggressive malignant behavior of thymic neoplasms regardless of tumor subtype. EGFR is moderately expressed in thymic neoplasms, and therapeutic targeting EGFR in thymomas warrants further investigation.

Quality Assurance

1493 Assessment of Specimen Adequacy in Bronchial Washings and Brushings: Correlation of Diagnostic Yield with Number of Benign Bronchial Epithelial Clusters, Bronchoscopic Findings and Tumor Size

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Background: Bronchial washings (BW) and brushings (BB) are valuable in the diagnosis of lung neoplasia. Adequacy is a challenging issue in nongynecologic cytology; in negative bronchial cytology, specimen adequacy is not well defined and a large number of well-preserved bronchial epithelium is recommended. The purpose of this study is to determine if the number of benign bronchial epithelial clusters (BEC), bronchoscopy visualization of lesion, or tumor size correlates with positive diagnosis.

Design: We reviewed 107 sequential bronchial cytology specimens (48 BB, 59 BW) and corresponding biopsy (Bx) from 54 different patients, obtained between Jan.-Jun. 2003. All patients have biopsy proven lung carcinoma. We defined BEC as a group of ≥3-10 well-preserved respiratory epithelial cells. Cytology slides were reviewed and correlated with histology. According to number of BEC, cases were subdivided into two groups: category I <10 BEC, II ≥10 BEC. Sample quality (poor fixation, inflammation, blood or mucous) was recorded. Patient records were reviewed for bronchoscopic findings and tumor size.

Results: Number of BEC, bronchoscopic findings and tumor size are summarized in table I. The most important predictor of positive cytology was bronchoscopic visualization of lesion in BB (P<0.001) and lack of visualization in BW (P <0.04). The number of BEC and tumor size did not correlate with positive diagnosis in either BB or BW. When compared to BB, BW is more likely to have positive diagnosis in non-visualized lesion (10 versus 5 cases). Bronchoscopy findings for 4 cases and tumor size for 2 cases were not available. Drying artifact was present in most BB smears. Blood, inflammation, or mucous didn't preclude a diagnosis in any case.

Conclusions: 1- In this limited study there is no correlation between the number of BEC and diagnostic yield. 2- Bronchoscopic visualization of lesion is predictive of positive diagnosis. BB is most diagnostic in visualized lesions and BW in non-visualized lesions. Utilization of bronchoscopic findings may allow for specimen enrichment by the bronchoscopist and/or specimen triage by the cytology lab.

BEC number, bronchoscopic finding and tumor size in BB and BW

	# BEC		Bronchoscopic finding		Tumor size	
	< 10	≥10	Lesion	No lesion	< 3 cm	≥3 cm
BB (n=48)						
Positive(n=15)	5	10	9	5	1	12
Negative(n=26)	8	18	2	23	5	19
BW(n=59)						
Positive(n=17)	11	6	6	10	3	12
Negative(n=28)	23	5	3	24	6	20
	BB P value=NS		BB P< 0.001		BB P= NS	
	BW P=NS		BW P< 0.04		BW P=NS	

1494 Efficacy of On-Site Specimen Adequacy Evaluation of Image-Guided Non-Thyroid Fine and Core Needle Biopsies

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Background: Cytology labs upon request provide on-site specimen adequacy evaluations assuming that they improve diagnostic yield. So far, the literature on this issue has been controversial and the cost-effectiveness of this practice remains unclear. Therefore, we have examined our own experience to assess its efficacy.

Design: Cytology reports on all image-guided non-thyroid fine needle (FNA) and core biopsies from January through June 2005 were retrieved. The specimens were divided into two categories: those with and without adequacy evaluations. Organ site, type of specimen (FNA or core), and final cytologic diagnosis were recorded for all specimens. For those with evaluations, the result of evaluation, and number of passes were recorded.

Results: 250 specimens were accrued during the study period from kidney (16), liver (42), lung (50), pancreas (51), retroperitoneum (7), and soft tissue (84). 204 were FNA, 38 were core, and 8 had both. 136 had adequacy evaluations and 114 did not. A definitive positive diagnosis was rendered on 96 (38%), i.e. 66 (49%) of the 136 with evaluations and 30 (26%) of the 114 without (P<0.001 by chi-square). When we stratified the analysis by organ, on-site evaluations significantly improved the diagnostic yield (as compared to those without evaluations) for liver (75% v 10%, P<0.001) and lung (67% v 30%, P=0.01), but not for kidney (64% v 60%, P=0.89), pancreas (13% v 11%, P=0.83), retroperitoneum (20% v 100%, P=0.05), or soft tissue (41% v 27%, P=0.19). No difference was noted in the total number of passes between the 99 specimens that were deemed adequate on evaluation versus the 37 deemed inadequate (2.72 v 2.76, P=0.89 by t test). Of the specimens deemed adequate at on-site evaluations, 77 (78%) were adequate on the first pass and 90 (90%) on the first two, but more passes were obtained in 55 (56%) either for ancillary studies or just to assure adequacy. Of the specimens deemed inadequate at on-site evaluations, 35 (95%) procedures stopped after 4 passes.

Conclusions: On-site adequacy evaluations were helpful for liver and lung but not for tumors of other organs. Since 90% of the adequate materials were obtained on the first two passes and 95% procedures stopped after 4 passes regardless of the adequacy result, on-site evaluations should be used selectively for the purpose of minimizing the number of passes instead of increasing the diagnostic yield.

1495 A Practical Multi-Step Approach To Reduce Errors in Surgical Pathology Specimen Handling and Processing

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Background: In recent years, medical errors and patient safety have become pressing national health care problems. In 2003 our anatomic pathology service experienced a sentinel event resulting from transposition of two breast core biopsies and subsequent inappropriate surgery. We report a multi-prong approach to prevent such errors.

Design: A multidisciplinary committee performed a thorough root-cause analysis and failure mode analysis following JCAHO guidelines. Following are some of the major changes implemented to ensure patient identity at each step of specimen handling: - Additional space was added to the accessioning area to improve workflow. - Batch entry was abandoned mandating individual handling of each specimen. - Besides the surgical number, two unique patient identifiers (name and date of birth) have been added to cassettes. - Cassette and slide labeling is now automated. - Each specimen is now placed in a separate plastic bin along with the requisition slip and cassettes. - Multiple patient identifiers are verified and dictated at the beginning of grossing. - High-risk cases (e.g. breast and prostate cores) are not handled back-to-back. - Pathologists receive one case per slide-tray. - Multiple monthly quality assurance indicators have been introduced to monitor the effectiveness of the corrective actions. - Continuous in-service education is given to residents, pathology assistants, laboratory assistants and histology technologists.

Results: Significant improvements were noted in quality control parameters compared between a 31-month period preceding and a 10-month period following implementation of the new specimen handling policy (see table).

	Average/ Month			Maximum/ Month		% Reduction in,	
	Cassettes Labeled	Error Events	Mislabeled Blocks	Error Events	Mislabeled Blocks	Error Events	Mislabeled Blocks
Before	7649.8	2.8	7.9	6	24		
After	8021	1.1	2.2	2	5	61%	72%
p-value		< 0.002	< 0.014				

Conclusions: Overall, the approach of maintaining the chain of patient identity at each step resulted in significant improvements. Specifically, non-batching, verification of multiple patient identifiers, physical separation of specimens and the culture of increased awareness have been particularly effective. The practice of proper specimen handling is being enforced with routine in-service education to eliminate the human error, still a significant risk factor despite marked improvement in the specimen handling process.